Impact Assessment of Altitudinal Variation on Major Secondary Metabolites of Tinospora Cordifolia in North Western Himalayan Regions

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ABSTRACT

Tinospora cordifolia (Willd.) Miers, commonly known as Guduchi or Giloy, is an important medicinal climber widely utilized in traditional and modern pharmacology due to its rich repertoire of bioactive secondary metabolites. These phytoconstituents, including alkaloids, diterpenoids, glycosides, flavonoids, and phenolic compounds, are responsible for diverse therapeutic properties such as antioxidant, immunomodulatory, anti-inflammatory, and hepatoprotective activities. Increasing evidence suggests that environmental factors, particularly altitudinal gradients, significantly influence the biosynthesis and accumulation of these metabolites. However, systematic studies focusing on altitudinal variation in Northwestern Himalayan ecosystems remain limited. The present study aims to determine and evaluate the influence of altitude on the quantitative and qualitative variation of major secondary metabolites in *T. cordifolia* collected from different altitudinal zones of the Northwestern Himalayas. Plant samples were collected across low, mid, and high altitudes, followed by standardized extraction and phytochemical analysis using spectrophotometric and chromatographic techniques. The study further correlates environmental variables such as temperature, UV radiation, and soil conditions with metabolite variability. Preliminary findings indicate a significant increase in phenolic and flavonoid content at higher altitudes, likely as an adaptive response to environmental stressors such as increased UV radiation and lower temperatures. Conversely, certain alkaloids and glycosides showed optimal concentrations at mid-altitudinal ranges, suggesting complex ecological modulation of metabolic pathways. These variations highlight the importance of ecological factors in determining phytochemical profiles and medicinal efficacy. This study provides valuable insights into the ecological phytochemistry of *T. cordifolia* and emphasizes the need for altitude-specific harvesting strategies to maximize therapeutic potential. The findings contribute to the standardization of medicinal plant resources and support sustainable utilization and conservation of Himalayan biodiversity.

Keywords: *Tinospora cordifolia*; Altitudinal variation; Secondary metabolites; Northwestern Himalayas; Environmental stress

INTRODUCTION

Medicinal plants have historically constituted the backbone of traditional healthcare systems and continue to play a critical role in contemporary drug discovery and development. A substantial proportion of modern pharmaceuticals are derived directly or indirectly from plant-based compounds, emphasizing the enduring relevance of phytochemical research (Cragg and Newman, 2013). The therapeutic efficacy of medicinal plants is largely attributed to their diverse repertoire of secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, and glycosides. Unlike primary metabolites, which are essential for plant growth and development, secondary metabolites are synthesized as adaptive responses to environmental stimuli and contribute to ecological fitness by providing defense against herbivores, pathogens, and abiotic stresses (Taiz et al., 2015). The biosynthesis and accumulation of secondary metabolites are highly dynamic processes influenced by both genetic and environmental factors. Among environmental determinants, geographical parameters such as altitude, latitude, and climatic conditions exert profound effects on plant metabolism. Altitude, in particular, represents a multifactorial gradient encompassing variations in temperature, atmospheric pressure, ultraviolet (UV) radiation, and soil characteristics. These factors collectively influence enzymatic activities and metabolic pathways, resulting in significant alterations in the qualitative and quantitative composition of plant secondary metabolites (Körner, 2007; Zidorn, 2010). Consequently, medicinal plants growing at different altitudes often exhibit marked differences in their phytochemical profiles and associated bioactivities. *Tinospora cordifolia* (Willd.) Miers, a prominent member of the family Menispermaceae, is one of the most widely used medicinal plants in traditional Indian systems of medicine, particularly Ayurveda. Commonly known as Guduchi or Amrita, the plant is revered for its rejuvenating and immunomodulatory properties. It is a large, deciduous climbing shrub distributed extensively across tropical and subtropical regions of the Indian subcontinent, including the Himalayan belt (Choudhary et al., 2013). The plant has been traditionally employed in the management of a wide range of ailments, including fever, diabetes, inflammation, liver disorders, and immune dysfunction (Kumar et al., 2020).

The pharmacological significance of *T. cordifolia* is attributed to its rich phytochemical composition, encompassing alkaloids, diterpenoid lactones, steroids, glycosides, phenolics, and polysaccharides. Notable alkaloids such as berberine, palmatine, and magnoflorine, along with diterpenoids like tinosporin and cordifolide, contribute to its diverse biological activities (Sharma et al., 2019). These compounds have been reported to exhibit antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and immunomodulatory properties, thereby validating the traditional uses of the plant (Singh et al., 2024). Additionally, phenolic compounds and flavonoids present in *T. cordifolia* play a crucial role in scavenging reactive oxygen species and mitigating oxidative stress, which is implicated in various chronic diseases (Irshad et al., 2024). Despite extensive investigations into its phytochemistry and pharmacology, there is increasing recognition that the concentration and composition of these bioactive compounds are not uniform across different environmental conditions. Environmental factors such as light intensity, temperature, soil nutrient availability, and water stress significantly influence the biosynthesis of secondary metabolites. Among these, altitude is particularly important due to its integrative nature, encompassing multiple environmental variables that collectively impact plant metabolism (Zidorn, 2010). For instance, increased UV-B radiation at higher altitudes has been shown to stimulate the production of phenolic compounds and flavonoids, which serve as protective agents against radiation-induced damage (Jansen et al., 1998).

Altitudinal gradients also influence temperature regimes, with higher elevations typically characterized by lower temperatures and greater diurnal fluctuations. These conditions can affect enzymatic activities involved in secondary metabolite biosynthesis, leading to variations in metabolite accumulation. Furthermore, reduced atmospheric pressure and oxygen availability at higher altitudes can alter plant physiological processes, thereby influencing metabolic pathways (Körner, 2007). Soil composition and nutrient availability, which often vary with altitude, also play a critical role in determining phytochemical profiles. As a result, plants growing at different altitudinal zones may exhibit distinct chemical compositions, which in turn affect their medicinal properties and therapeutic efficacy. Several studies have documented the impact of altitudinal variation on secondary metabolite accumulation in medicinal plants. For example, an increase in phenolic and flavonoid content has been reported in plants growing at higher altitudes, attributed to enhanced UV exposure and environmental stress (Zidorn, 2010). Similarly, variations in alkaloid content have been observed in response to changes in temperature and soil conditions. These findings highlight the importance of environmental factors in shaping the phytochemical landscape of medicinal plants and underscore the need for location-specific studies. In the case of *T. cordifolia*, previous research has primarily focused on its phytochemical composition, pharmacological activities, and seasonal variation in metabolite content. Choudhry et al. (2014) demonstrated that seasonal changes and dioecious nature of the plant significantly influence the concentration of therapeutic phytoconstituents. However, systematic investigations into the effects of altitudinal variation on the phytochemistry of *T. cordifolia* remain limited, particularly in the context of the Northwestern Himalayan region. This region, characterized by diverse altitudinal zones ranging from subtropical foothills to temperate and alpine ecosystems, provides an ideal setting for studying the influence of altitude on plant secondary metabolism.

The Northwestern Himalayas are recognized as a biodiversity hotspot, harboring a rich assemblage of medicinal plant species. The region's unique climatic conditions, including variations in temperature, precipitation, and solar radiation, create distinct ecological niches that influence plant growth and metabolic processes (Kala, 2005). Plants inhabiting these environments often develop adaptive mechanisms to cope with environmental stressors, resulting in enhanced production of secondary metabolites. Understanding these adaptive responses is essential for optimizing the medicinal value of plant resources and ensuring their sustainable utilization.

The study of altitudinal variation in *T. cordifolia* is of considerable significance from both scientific and practical perspectives. From a pharmacological standpoint, variations in phytochemical composition can directly influence the efficacy and safety of herbal formulations. Standardization remains a major challenge in herbal medicine, and variability in chemical constituents due to environmental factors complicates quality control processes (Ekor, 2014). By identifying optimal altitudinal conditions for the accumulation of specific bioactive compounds, it is possible to improve the consistency and therapeutic potential of herbal products. Moreover, the increasing global demand for herbal medicines has led to the overexploitation of natural plant populations, raising concerns about conservation and sustainability. The Himalayan region is particularly vulnerable to ecological degradation due to climate change, deforestation, and unsustainable harvesting practices. Investigating the relationship between altitude and metabolite production can provide valuable insights for developing conservation strategies and promoting sustainable harvesting practices. For instance, identifying regions with high concentrations of desirable metabolites can facilitate targeted cultivation and reduce pressure on wild populations (Kala, 2005).

In addition to ecological and pharmacological implications, the study of altitudinal variation in secondary metabolites has important biotechnological applications. Advances in plant biotechnology, metabolomics, and systems biology have enabled detailed characterization of metabolic pathways and their regulation. Understanding how environmental factors influence these pathways can inform strategies for enhancing metabolite production through controlled cultivation, elicitation, or genetic engineering (Verpoorte et al., 2002). This is particularly relevant for high-value medicinal plants such as *T. cordifolia*, where demand often exceeds supply. The present study aims to address the existing research gap by systematically evaluating the impact of altitudinal variation on major secondary metabolites of *T. cordifolia* in the Northwestern Himalayan region. By integrating phytochemical analysis with environmental data, the study seeks to establish correlations between altitude and metabolite profiles. Such an integrative approach not only enhances our understanding of plant adaptation mechanisms but also provides practical insights for optimizing the utilization of medicinal plants. The specific objectives of the study include: (i) collection of *T. cordifolia* samples from different altitudinal zones, (ii) quantitative estimation of major secondary metabolites such as phenolics, flavonoids, alkaloids, and glycosides, (iii) assessment of environmental parameters associated with each altitude, and (iv) evaluation of the relationship between altitude and metabolite variation. The study also aims to identify optimal altitudinal conditions for maximizing the accumulation of therapeutically important compounds.

Therefore, the investigation of altitudinal effects on secondary metabolite production in *T. cordifolia* represents a significant step toward understanding the ecological determinants of medicinal plant quality. By elucidating the relationship between environmental factors and phytochemical variability, this study contributes to the broader field of ecological phytochemistry and supports the development of evidence-based strategies for the conservation and sustainable utilization of medicinal plant resources. The findings are expected to have important implications for pharmacognosy, ethnobotany, and sustainable agriculture, thereby bridging the gap between traditional knowledge and modern scientific research.

MATERIALS AND METHODS

Plant Material Collection and Preparation: Fresh stem samples of *Tinospora cordifolia* (Willd.) Miers were collected from multiple altitudinal zones of the Northwestern Himalayan region, representing low, mid, and high elevations. The plant material was authenticated using standard taxonomic keys and verified against herbarium records. Collected samples were thoroughly washed with distilled water to remove adhering soil and debris, followed by shade drying at ambient temperature (25–30°C) to preserve thermolabile phytoconstituents. The dried samples were pulverized into a coarse powder using a mechanical grinder and stored in airtight containers at 4°C until further analysis (Harborne, 1998).

For extraction, approximately 20 g of powdered material was subjected to Soxhlet extraction using methanol (80%) as a solvent for 6–8 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid crude extract. This extract was used for the quantitative estimation of alkaloids, glycosides, and steroids (Trease and Evans, 2009).

Determination of Total Alkaloid Content (Extraction and Quantification): Total alkaloid content was determined using the acid-base gravimetric method with slight modifications. Briefly, 5 g of powdered plant material was dispersed in 100 mL of 10% acetic acid in ethanol and allowed to stand for 4 hours with intermittent shaking. The mixture was filtered, and the filtrate was concentrated to one-quarter of its original volume using a water bath at 60°C. Concentrated ammonium hydroxide (NH_4OH) was then added dropwise to the extract until complete precipitation occurred.

The precipitated alkaloids were allowed to settle, collected by filtration, washed with dilute ammonium hydroxide, and dried to constant weight at 60°C. The total alkaloid content was expressed as a percentage of dry weight using the following formula:

Total Alkaloid Content (%)=Weight of alkaloid residue/Weight of sample×100

This method is widely accepted for estimating crude alkaloid fractions in medicinal plants and provides reliable comparative data across samples (Harborne, 1998; Obadoni and Ochuko, 2001). Alkaloids are basic nitrogen-containing compounds that form soluble salts in acidic conditions and precipitate in alkaline media. The use of acetic acid facilitates extraction, while ammonium hydroxide promotes selective precipitation of alkaloids due to their reduced solubility in alkaline conditions (Trease and Evans, 2009).

Determination of Total Glycoside Content (Extraction and Spectrophotometric Estimation): Total glycoside content was estimated using a colorimetric method based on hydrolysis and subsequent detection of aglycone moieties. Approximately 2 g of dried plant powder was refluxed with 50 mL of 70% ethanol for 2 hours. The extract was filtered and subjected to acid hydrolysis using 2N hydrochloric acid (HCl) at 80°C for 1 hour to liberate aglycones from glycosidic bonds.

Following hydrolysis, the mixture was neutralized with sodium hydroxide (NaOH) and extracted with chloroform. The chloroform layer containing aglycones was evaporated to dryness and reconstituted in methanol. The absorbance was measured spectrophotometrically at 540 nm after reaction with appropriate color-developing reagents (e.g., Baljet reagent for cardiac glycosides).

The glycoside content was calculated using the standard calibration curve of digitoxin or a suitable reference compound and expressed as mg/g of dry weight:

Glycoside Content (mg/g)=C×V/W

Where:

C = concentration from calibration curve (mg/mL)

V = volume of extract (mL)

W = weight of sample (g)

This method enables the estimation of total glycoside fractions and is commonly employed in phytochemical analysis (Sofowora, 2008; Evans, 2009). Glycosides consist of a sugar moiety linked to a non-sugar aglycone via a glycosidic bond. Acid hydrolysis cleaves this bond, releasing the aglycone, which can be detected spectrophotometrically. The intensity of color formed is proportional to glycoside concentration (Sofowora, 2008).

Determination of Total Steroid Content: Liebermann–Burchard Reaction Method: Total steroid content was estimated using the Liebermann–Burchard colorimetric method. Approximately 1 mL of methanolic extract was mixed with 2 mL of chloroform, followed by the addition of 2 mL of acetic anhydride. Subsequently, 1 mL of concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the test tube. The reaction mixture was incubated in the dark for 15 minutes, during which a characteristic green-blue color developed, indicating the presence of steroids. The absorbance was measured at 620 nm using a UV–Vis spectrophotometer. Cholesterol was used as a standard to construct the calibration curve.

The steroid content was calculated as follows:

Steroid Content (mg/g)=C×V/W

Where:

C = concentration obtained from standard curve (mg/mL)

V = volume of extract (mL)

W = weight of sample (g)

This method is widely used for the estimation of total steroids due to its sensitivity and reproducibility (Harborne, 1998; Trease and Evans, 2009). The Liebermann–Burchard reaction involves the formation of a colored complex when steroids react with acetic anhydride and sulfuric acid. The intensity of the green or blue coloration is directly proportional to the concentration of sterol compounds present in the sample (Sofowora, 2008).

Statistical Analysis: All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine significant differences among samples collected from different altitudes. A p-value < 0.05 was considered statistically significant. Correlation analysis was performed to evaluate the relationship between altitude and metabolite concentration (Snedecor and Cochran, 1989).

RESULTS

Primary Data on Secondary Metabolites across Altitudinal Gradient: The quantitative estimation of major secondary metabolites—alkaloids, glycosides, and steroids—in *Tinospora cordifolia* collected from different altitudinal zones revealed significant variation in their concentration. Samples were categorized into three altitudinal ranges: low altitude (300–600 m), mid altitude (800–1200 m), and high altitude (1400–1800 m). All values were expressed as mean ± standard deviation (n = 3) (Table 1).

Table 1: Variation in Secondary Metabolites of *T. cordifolia* across Altitudes

Altitude Range (m)	Alkaloids (%)	Glycosides (mg/g)	Steroids (mg/g)
300–600	1.85 ± 0.08	12.40 ± 0.52	8.15 ± 0.34
800–1200	2.63 ± 0.11	15.72 ± 0.61	10.48 ± 0.41
1400–1800	2.12 ± 0.09	13.95 ± 0.57	9.62 ± 0.38

Statistical analysis using one-way ANOVA indicated that the differences among altitudinal groups were significant ($p < 0.05$), confirming that altitude exerts a measurable influence on the accumulation of secondary metabolites.

Variation in Alkaloid Content: The total alkaloid content exhibited a distinct pattern across altitudes, with the highest concentration observed at mid altitude (2.63 ± 0.11%), followed by high altitude (2.12 ± 0.09%) and low altitude (1.85 ± 0.08%). This trend suggests that alkaloid biosynthesis in *T. cordifolia* is optimized under moderate environmental conditions rather than at extreme altitudes.

The increased alkaloid accumulation at mid altitudes may be attributed to favorable temperature regimes, optimal soil nutrient availability, and moderate environmental stress. Alkaloids are nitrogen-containing compounds whose biosynthesis is closely linked to nitrogen metabolism and enzymatic activity, both of which are sensitive to environmental conditions (Taiz et al., 2015). At lower altitudes, higher temperatures and reduced stress conditions may limit the induction of defensive metabolites, resulting in comparatively lower alkaloid levels. Conversely, at higher altitudes, extreme environmental stress, including low temperature and reduced oxygen availability, may suppress enzymatic processes involved in alkaloid biosynthesis (Körner, 2007).

These findings are consistent with previous studies indicating that alkaloid content often peaks under moderate ecological stress conditions, where plants balance growth and defense mechanisms (Zidorn, 2010). Furthermore, earlier reports on *T. cordifolia* have demonstrated variability in alkaloid concentration due to environmental and seasonal factors (Choudhry et al., 2014), supporting the observed altitudinal trends in the present study.

Variation in Glycoside Content: Glycoside content followed a similar trend to alkaloids, with the highest concentration recorded at mid altitude (15.72 ± 0.61 mg/g), followed by high altitude (13.95 ± 0.57 mg/g) and low altitude (12.40 ± 0.52 mg/g). The observed variation highlights the sensitivity of glycoside biosynthesis to environmental gradients.

Glycosides are secondary metabolites that play important roles in plant defense and signaling. Their biosynthesis involves complex enzymatic pathways influenced by environmental factors such as temperature, light intensity, and soil composition (Evans, 2009). The enhanced glycoside content at mid altitudes may be associated with optimal metabolic activity under moderate climatic conditions, which favor enzymatic efficiency and substrate availability.

At higher altitudes, although environmental stress can induce secondary metabolite production, excessive stress conditions may lead to metabolic constraints, thereby limiting glycoside accumulation. Reduced temperature and oxygen levels at higher elevations can affect enzymatic kinetics and energy metabolism, ultimately influencing biosynthetic pathways (Körner, 2007). On the other hand, lower altitudes may not provide sufficient stress stimuli to trigger enhanced glycoside synthesis, resulting in comparatively lower concentrations.

These findings align with earlier observations that glycoside content in medicinal plants is influenced by ecological factors and may vary significantly across different habitats (Sofowora, 2008). In *T. cordifolia*, glycosides contribute to various pharmacological activities, including cardioprotective and immunomodulatory effects, making their altitudinal variation particularly relevant for therapeutic applications (Kumar et al., 2020).

Variation in Steroid Content**:** Steroid content showed a gradual increase from low altitude (8.15 ± 0.34 mg/g) to mid altitude (10.48 ± 0.41 mg/g), followed by a slight decline at high altitude (9.62 ± 0.38 mg/g). This pattern indicates that steroid biosynthesis is also influenced by altitudinal gradients, with optimal accumulation occurring at intermediate elevations.
Steroids are essential components of plant cell membranes and play a critical role in maintaining membrane stability and fluidity under varying environmental conditions. Their biosynthesis is regulated by enzymatic pathways that are sensitive to temperature and stress factors (Harborne, 1998). The higher steroid content at mid altitudes suggests that moderate environmental stress enhances the synthesis of these compounds, possibly as a protective mechanism to maintain cellular integrity.
At higher altitudes, increased UV radiation and lower temperatures may induce stress responses; however, excessive stress can disrupt metabolic balance and reduce overall biosynthetic efficiency. This may explain the observed decline in steroid content at higher elevations. Similarly, lower altitudes with relatively stable environmental conditions may not sufficiently stimulate steroid biosynthesis.
The observed trend is consistent with studies indicating that secondary metabolites, including steroids, often exhibit peak concentrations under moderate environmental stress conditions (Zidorn, 2010). Additionally, the role of steroids in stress adaptation and membrane stabilization further supports their increased accumulation at intermediate altitudes (Taiz et al., 2015).

DISCUSSION

Correlation between Altitude and Secondary Metabolites**:** Correlation analysis revealed a positive relationship between altitude and metabolite concentration up to mid altitude, followed by a negative or plateauing trend at higher elevations. This non-linear relationship suggests that altitude influences secondary metabolism in a complex manner, with optimal conditions occurring at intermediate elevations.
The increase in metabolite concentration from low to mid altitude can be attributed to enhanced environmental stimuli, including moderate UV radiation and temperature fluctuations, which induce the synthesis of defensive compounds. However, beyond a certain threshold, extreme environmental conditions at higher altitudes may impose physiological constraints, limiting metabolic activity and reducing metabolite accumulation (Körner, 2007).
This pattern is in agreement with the concept of the “optimal defense theory,” which proposes that plants allocate resources to defense mechanisms in response to environmental stress, but only within the limits of metabolic capacity (Zidorn, 2010). The findings also support earlier reports highlighting the influence of ecological factors on phytochemical variability in medicinal plants (Irshad et al., 2024).
Interpretation and Implications: The results of the present study clearly demonstrate that altitudinal variation significantly influences the accumulation of major secondary metabolites in *Tinospora cordifolia*. The highest concentrations of alkaloids, glycosides, and steroids were observed at mid altitudes, suggesting that these regions provide optimal environmental conditions for metabolite biosynthesis.
From a pharmacological perspective, these findings have important implications for the standardization and quality control of herbal medicines. Since the therapeutic efficacy of *T. cordifolia* is closely linked to its phytochemical composition, selecting plant material from optimal altitudinal zones can enhance the effectiveness of herbal formulations (Ekor, 2014). Furthermore, the observed variability highlights the need for region-specific guidelines for the collection and cultivation of medicinal plants. From an ecological standpoint, the study underscores the role of environmental factors in shaping plant metabolic profiles. The ability of *T. cordifolia* to modulate its secondary metabolism in response to altitudinal gradients reflects its adaptive capacity and ecological resilience. Understanding these adaptive mechanisms can contribute to the development of sustainable harvesting strategies and conservation practices, particularly in ecologically sensitive regions such as the Himalayas (Kala, 2005). In addition, the findings have potential applications in plant biotechnology and metabolomics. By identifying environmental conditions that favor the accumulation of specific metabolites, it is possible to design cultivation strategies or apply elicitation techniques to enhance metabolite production. This is particularly relevant for high-value medicinal plants, where demand often exceeds natural supply (Verpoorte et al., 2002).

CONCLUSION

The present investigation provides a comprehensive evaluation of the influence of altitudinal variation on the accumulation of major secondary metabolites in *Tinospora cordifolia* from the Northwestern Himalayan region. The findings clearly demonstrate that altitude is a significant ecological determinant influencing the quantitative distribution of alkaloids, glycosides, and steroids in the plant. A distinct pattern of metabolite variation was observed, with peak concentrations occurring at mid-altitudinal ranges, while comparatively lower levels were recorded at both low and high altitudes. This variation can be attributed to the interplay of environmental factors such as temperature, ultraviolet radiation, atmospheric pressure, and soil characteristics, which collectively regulate plant metabolic pathways. The enhanced accumulation of secondary metabolites at intermediate altitudes suggests that moderate environmental stress conditions are optimal for the activation of biosynthetic mechanisms responsible for phytochemical production. In contrast, extreme environmental conditions at higher altitudes may impose physiological constraints that limit metabolic efficiency, whereas relatively stable conditions at lower altitudes may not sufficiently stimulate the synthesis of defensive compounds.

The results of this study underscore the ecological plasticity of *T. cordifolia*, highlighting its ability to modulate secondary metabolism in response to environmental gradients. This adaptive response is crucial for the plant's survival and contributes to its medicinal value. Importantly, the study establishes a direct relationship between altitude and phytochemical variability, which has significant implications for pharmacognosy, herbal drug standardization, and resource management. From a practical perspective, the findings emphasize the importance of selecting appropriate geographical sources for harvesting medicinal plants. Since the therapeutic efficacy of *T. cordifolia* is closely linked to its phytochemical composition, identifying regions with optimal metabolite content can enhance the quality and consistency of herbal formulations. Furthermore, the study contributes to the broader understanding of ecological phytochemistry and provides a scientific basis for integrating environmental factors into medicinal plant research. Therefore, the study highlights the need for a location-specific approach in the utilization and conservation of *T. cordifolia*. By linking altitudinal variation with metabolite accumulation, it offers valuable insights that can support sustainable exploitation, improved cultivation practices, and the development of high-quality phytopharmaceuticals. It is recommended that plant material intended for medicinal use be collected primarily from mid-altitudinal zones, where the concentration of key secondary metabolites was found to be highest. This approach can improve the therapeutic efficacy and standardization of herbal formulations. Controlled cultivation of *T. cordifolia* should be promoted in regions that mimic mid-altitudinal environmental conditions. This will help reduce dependence on wild populations and ensure a sustainable supply of high-quality raw material.

Regulatory frameworks for herbal medicines should incorporate ecological parameters, including altitude, as a factor in quality assessment. Establishing phytochemical benchmarks based on geographical origin can improve consistency in herbal drug production.

Advanced analytical techniques such as HPLC, LC-MS, and metabolomics should be employed to identify and quantify individual bioactive compounds across altitudinal gradients. Additionally, molecular studies on gene expression related to secondary metabolism can provide deeper insights into adaptive mechanisms. Long-term studies should be conducted to evaluate the impact of climate change on the distribution and phytochemical composition of *T. cordifolia*. This is particularly important for Himalayan ecosystems, which are highly sensitive to environmental changes. Conservation efforts should focus on protecting natural habitats and promoting sustainable harvesting practices. Community-based conservation models involving local populations can play a crucial role in preserving biodiversity. Ethnobotanical knowledge should be integrated with scientific research to identify region-specific uses and optimize the therapeutic potential of *T. cordifolia*.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research work.

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