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Sustainable utilisation of *Tylophora indica*, an endangered medicinal plant

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Abstract
Tylophora indica (Burm. f.) Merr., a perennial climber from the Apocynaceae family, is a critically endangered medicinal plant renowned for its immense therapeutic value, particularly in traditional Ayurvedic medicine for treating respiratory ailments like asthma and bronchitis. Its pharmacological properties are primarily attributed to a unique class of bioactive compounds, the phenanthroindolizidine alkaloids, with tylophorine being the most significant. Rampant over-exploitation from wild habitats to meet commercial demand, coupled with its inherent constraints of low seed viability and poor germination rates, has pushed this species to the brink of extinction. This underscores an urgent need for a multifaceted conservation strategy. This review comprehensively explores the integrated approaches essential for the sustainable utilisation of *T. indica*. We discuss the critical role of in-situ conservation through habitat protection and the establishment of medicinal plant conservation areas. Furthermore, we emphasise the paramount importance of advanced ex-situ strategies, including biotechnological interventions like plant tissue culture for rapid clonal propagation, synthetic seed technology, and cell suspension cultures for continuous biomass and alkaloid production. Ultimately, a synergistic model that combines strict wild resource management with the adoption of modern cultivation and biotechnology techniques is proposed as the only viable pathway to ensure the long-term availability of this valuable genetic resource without compromising its natural populations.

Keywords: *Tylophora indica*, endangered medicinal plant, sustainable utilisation, conservation strategies, plant tissue culture, phenanthroindolizidine alkaloids, micropropagation, *in-vitro* conservation

Introduction

The plant kingdom has been an indispensable source of medicine for millennia, forming the foundation of traditional healthcare systems like Ayurveda, Siddha, and Unani. Even in modern pharmacopoeia, a significant proportion of drugs are either directly derived from or inspired by plant-based compounds. Among this vast array of medicinal flora, *Tylophora indica* (Burm. f.) Merr. (Family: Apocynaceae) holds a position of significant importance. Commonly known as "Antamul" or "Indian Ipecacuanha," it is a perennial, branching climber indigenous to the plains and hill ranges of the Indian subcontinent.

The therapeutic reputation of *T. indica* is primarily built upon its efficacy in treating respiratory disorders. Its leaves have been used for centuries in Ayurvedic medicine as a potent anti-asthmatic, expectorant, and anti-inflammatory agent. Scientific validation has confirmed its bronchodilatory and anti-allergic properties, effectively mitigating the symptoms of asthma, bronchitis, and allergic rhinitis. Beyond its respiratory applications, research has unveiled a broader spectrum of pharmacological activities, including immunomodulatory, anti-cancer, anti-diabetic, and anti-microbial properties. These diverse biological activities are attributed to a suite of bioactive secondary metabolites, most notably the phenanthroindolizidine alkaloids, with tylophorine, tylophorinine, and tylophorinidine being the principal compounds responsible for its medicinal effects.

Paradoxically, the very properties that make *T. indica* so valuable are also the cause of its peril. The escalating demand for its raw material in the pharmaceutical and herbal drug industries has led to uncontrolled and indiscriminate harvesting from its natural wild habitats. This commercial over-exploitation is compounded by several biological constraints intrinsic to the plant. *T. indica* exhibits low reproductive efficiency, characterised by poor seed set, low seed viability, and erratic germination patterns, which severely limit its natural regeneration capacity. Furthermore, habitat destruction due to urbanisation, deforestation, and agricultural expansion has fragmented its populations, reducing their genetic diversity and resilience.

As a result of these synergistic pressures, *Tylophora indica* has been categorised as a "Critically Endangered" species by the International Union for Conservation of Nature (IUCN) and is listed in Appendix II of the Convention on International Trade in Endangered Species (CITES), which regulates its international trade. This alarming status presents a critical dilemma: how to balance the increasing medicinal demand with the imperative of conserving a threatened species.

Therefore, shifting from wild collection to sustainable utilisation practices is no longer an option but a necessity. This requires a comprehensive strategy that integrates in-situ conservation (protecting the plant in its natural ecosystem) with advanced ex-situ conservation techniques. The latter, particularly plant biotechnology, offers powerful tools. *In vitro* micropropagation can produce a large number of genetically uniform, disease-free plants year-round, independent of seasonal constraints. Techniques like cell and organ culture can be explored not only for conservation but also for the sustainable production of valuable alkaloids through bioreactors, thereby alleviating the pressure on wild stocks.

This review aims to synthesise the current knowledge and propose a coherent framework for the sustainable utilisation of *Tylophora indica*. It will explore the causes behind its endangerment, evaluate the strengths and limitations of

existing conservation methods, and highlight the pivotal role of biotechnological advancements in ensuring that this ancient medicinal treasure is preserved for future generations while meeting contemporary healthcare needs.

Methods

This review employed a systematic approach to gather, evaluate, and synthesise the available scientific literature on the conservation and biotechnological advancements for *Tylophora indica*. The aim was to provide a comprehensive analysis of the strategies for its sustainable utilisation.

1. Literature Search Strategy

A comprehensive literature search was conducted using major scientific databases, including Scopus, Web of Science, PubMed, Google Scholar, and specialised botanical databases like JSTOR Plant Science. The search was performed using a combination of keywords and phrases such as *"Tylophora indica"*, *"Antamul"*, *"endangered medicinal plant"*, *"conservation"*, *"micropropagation"*, *"in vitro culture"*, *"somatic embryogenesis"*, *"alkaloid production"*, *"tylophorine"*, *"cryopreservation"*, and *"synthetic seeds"*. Boolean operators (AND, OR) were used to refine the search. The search was not restricted by date to include foundational studies, but priority was given to articles from the last two decades to reflect current technologies.

2. Study Selection and Data Extraction

The initial search yielded a large number of records. Titles and abstracts were screened for relevance to the core themes of: (1) the causes of endangerment of *T. indica*, (2) in-situ and ex-situ conservation efforts, and (3) biotechnological interventions for its propagation and metabolite production. Full-text articles of relevant studies were then obtained and thoroughly reviewed. Data extracted from each selected study included the type of study (review, original research), specific conservation or biotechnological method employed, explant type used, culture media composition, growth regulators used, key findings related to propagation efficiency (e.g., shooting response, rooting percentage), and alkaloid yield where applicable.

3. Data Synthesis and Analysis

Given the narrative and methodological diversity of the included studies (e.g., ecological surveys, tissue culture experiments, phytochemical analyses), a meta-analysis was not feasible. Instead, a systematic narrative synthesis approach was adopted. The findings were categorised into major themes:

- **Threats and Conventional Conservation:** Synthesising data on population status, threats, and traditional horticultural practices.
- ***In-vitro* Propagation:** Compiling and comparing protocols for micropropagation via nodal segments, shoot tips, and leaf explants, analysing the role of various plant growth regulators (PGRs).
- **Secondary Metabolite Production:** Reviewing studies on callus, cell suspension, and hairy root cultures for the *in-vitro* production of tylophorine and related alkaloids.
- **Advanced Storage Technologies:** Evaluating methods for long-term germplasm storage, including synthetic seed technology and cryopreservation.

The synthesis aimed to identify the most effective and reproducible protocols, highlight research gaps, and propose an integrated model for sustainable use.

Results

The systematic review of literature revealed a significant body of research focused on addressing the critical endangerment of *Tylophora indica* through both conventional and advanced biotechnological methods.

1. Analysis of Threats and Limitations of Conventional Methods

The primary threat to *T. indica* is unequivocally identified as overharvesting from wild populations to supply raw material to the pharmaceutical industry. Ecological studies note that its natural habitats in deciduous forests are increasingly fragmented. Furthermore, the plant's biological constraints severely hinder conservation through traditional means:

- **Seed Propagation:** Studies consistently report very low seed viability (often below 30%) and poor germination rates (10-20% under natural conditions), which can take several weeks. Pre-treatment with gibberellic acid (GA_3) has been shown to improve germination percentages to 40-60%, but this remains insufficient for large-scale replenishment.
- **Vegetative Propagation:** While possible through stem cuttings, the success rate is highly variable and dependent on season and rooting hormones. This method is also not scalable to produce the millions of plants required to meet market demand and repopulate wild habitats.

2. Success of *In-vitro* Micropropagation

Biotechnological approaches, particularly micropropagation, have demonstrated remarkable success and represent the most promising solution. Research has optimised protocols using various explants.

- **Explant Selection:** Nodal segments containing axillary buds are established as the most responsive and genetically stable explant source, outperforming shoot tips and leaf segments.
- **Culture Media and Plant Growth Regulators (PGRs)**

Shoot Initiation and Proliferation: Murashige and Skoog (MS) medium is universally effective. A cytokinin, primarily 6-Benzylaminopurine (BAP), is essential for breaking bud dormancy and promoting shoot multiplication. The optimal concentration ranges from 1.0 to 3.0 mg/L. Studies show that the combination of BAP (2.0 mg/L) with a low concentration of an auxin like Indole-3-acetic acid (IAA) or α-Naphthalene acetic acid (NAA) (0.1-0.5 mg/L) often results in a synergistic effect, producing the highest number of shoots (8-12 shoots per node) within 4-6 weeks.

Rooting: Individual micro-shoots are effectively rooted ex vitro by treating the base with a quick dip in a high

concentration of auxin (e.g., 500 mg/L IAA or IBA for 1-2 minutes) before planting in sterile soil substrate, with reported success rates of 80-90%. *In-vitro* rooting on half-strength MS medium supplemented with IBA (1.0-2.0 mg/L) is also successful.

Acclimatisation: A critical phase with a high success rate (over 85%) achieved by gradually transferring plantlets from high-humidity culture conditions to the greenhouse and eventually to the field.

3. Biotechnological Production of Secondary Metabolites

A key strategy for reducing pressure on wild plants is the *in vitro* production of valuable alkaloids.

- **Callus and Cell Suspension Cultures:** Callus is induced from leaf explants on MS medium supplemented with the auxin 2,4-Dichlorophenoxyacetic acid (2,4-D). Suspension cultures derived from this callus show growth and alkaloid production that are highly dependent on PGR manipulation, nutrient stress (e.g., sucrose or nitrate concentration), and elicitation (e.g., using jasmonic acid or fungal elicitors). Some studies report tylophorine yields in cell cultures that are comparable to, or even exceed, those found in field-grown leaves, demonstrating the feasibility of this approach for "pharming" bioactive compounds.

- **Hairy Root Cultures:** Transformation using *Agrobacterium rhizogenes* to generate hairy roots is a particularly successful strategy. These genetically transformed roots are fast-growing, genetically stable, and known for high production of secondary metabolites. Research confirms that *T. indica* hairy root cultures exhibit significant levels of tylophorine production, making them an excellent platform for scalable bioreactor production.

4. Advanced Germplasm Conservation Techniques

For the long-term, cost-effective preservation of genetic resources, advanced technologies have been developed.

- **Synthetic Seed Technology:** Encapsulation of somatic embryos or, more commonly, unipolar shoot tips in a calcium alginate matrix is highly effective. These synthetic seeds can be stored at 4°C for short-to-medium term (1-3 months) and still exhibit high regrowth rates (~70-80%) upon germination on culture media. This is invaluable for the exchange of germplasm and the creation of working conservation banks.

- **Cryopreservation:** Protocols using the vitrification technique, where shoot tips are treated with a cryoprotectant like PVS2 before immersion in liquid nitrogen (-196°C), have been successfully established. Post-thaw recovery rates, while lower than other methods (around 40-50% in reported studies), provide a means for the truly long-term preservation of genetic diversity without the risk of somaclonal variation or the need for frequent subculturing.

5. Integrated Conservation Model

The results collectively advocate for an Integrated Conservation and Utilisation Model (ICUM). This model proposes:

- **Strict in-situ protection** of existing wild populations in designated conservation areas.

- **Mass propagation** of genetically diverse plantlets via the optimised micropropagation protocols to (a) supply farmers for organised cultivation and (b) for reintroduction and habitat restoration programs.

- **Establishment of *in vitro* metabolite production systems** (hairy roots, cell suspensions) to source alkaloids for industry, directly reducing the economic incentive for wild harvesting.

- **Creation of cryobanks** to preserve the full genetic portfolio of the species for future use.

Discussion

The critical endangerment of *Tylophora indica* presents a complex challenge at the intersection of biodiversity conservation, public health, and economic development. The findings of this review demonstrate that while the threats are severe, a robust arsenal of biotechnological tools exists to not only prevent its extinction but also create a sustainable pipeline for its medicinal compounds. This discussion interprets these results, weighing the efficacy of different strategies and situating them within a broader framework of medicinal plant conservation.

1. The Central Role of Biotechnology in Circumventing Biological Constraints

The fundamental limitation of conventional propagation for *T. indica* is its recalcitrant reproductive biology. The poor seed viability and germination are likely evolutionary traits that, while perhaps advantageous in its native niche, render it utterly vulnerable to commercial-scale demand. Micropropagation via nodal explants successfully bypasses this bottleneck entirely. The high efficacy of BAP in stimulating axillary bud break is well-documented in many woody perennials, as cytokinins antagonise apical dominance. The synergistic effect observed with low-dose auxins aligns with the established role of auxins in promoting cell division and vascular connection, facilitating the development of robust shoots. The success of ex vitro rooting is particularly significant from a commercial perspective, as it reduces the time and cost associated with *in vitro* rooting steps, streamlining the production of plantlets ready for acclimatisation. This high-throughput propagation is the cornerstone of any sustainable model, capable of supplying the quantity of planting material needed for systematic cultivation.

2. Beyond Propagation: *In-vitro* Factories for Metabolite Production

Perhaps the most paradigm-shifting outcome of this research is the development of *in vitro* systems for direct alkaloid production. While micropropagation addresses the supply of plants, it still requires land, time, and agricultural resources for cultivation. Cell suspension and, especially, hairy root cultures offer a more direct solution. Hairy roots, induced by the integration of *rol* genes from *Agrobacterium rhizogenes*,

are genetically stable and exhibit fast, hormone-independent growth. Their proven capacity to produce tylophorine suggests they can act as "bio-factories." This method decouples the production of the active pharmaceutical ingredient (API) from the plant itself. Industries could source tylophorine from a controlled, contaminant-free bioreactor process rather than from extracted wild plant material. This not only alleviates harvesting pressure but also offers the advantages of consistency, scalability, and year-round production independent of climatic conditions and seasonal variations that affect field-grown plants.

3. Ensuring Long-Term Genetic Integrity and Feasibility

The development of synthetic seeds and cryopreservation protocols addresses two different but critical aspects of conservation. Synthetic seeds (alginate-encapsulated shoot tips) are a practical tool for the medium-term storage and, crucially, the easy and sterile exchange of germplasm between research institutions, genebanks, and cultivators. This facilitates the preservation of a wider genetic pool. Cryopreservation, while technically more demanding and currently yielding lower recovery rates, is the ultimate insurance policy against genetic erosion. Placing germplasm in a state of suspended animation prevents the somaclonal variations that can accumulate during repeated subculturing in micropropagation. This ensures that the genetic diversity of *T. indica*, which is vital for its long-term adaptation and resilience to diseases and climate change, is preserved for future generations. The current ~50% recovery rate, while not ideal, is a strong foundation for further protocol refinement.

4. Challenges and Future Directions

Despite the promising results, several challenges remain before these biotechnological solutions can be fully implemented on a commercial scale.

- **Scaling Up:** While laboratory-scale protocols are well-established, scaling cell suspension or hairy root cultures to industrial-scale bioreactors (e.g., 1000L capacity) presents engineering challenges related to oxygen transfer, nutrient mixing, and shear stress that can affect growth and metabolite yield.

- **Economic Viability:** A thorough cost-benefit analysis is needed. The initial investment in bioreactor technology and the operational costs of running sterile cultures must be competitive with the cost of traditional cultivation and wild harvesting (which currently externalises the cost of biodiversity loss).

- **Metabolite Yield Optimisation:** While *in-vitro* systems produce alkaloids, consistently maximising yield is key to profitability. Future research must focus on advanced elicitation strategies (e.g., use of nano-elicitors), metabolic engineering, and precursor feeding to push yields to commercially viable levels.

- **Farmer and Industry Adoption:** A successful model requires buy-in from all stakeholders. This involves transferring micropropagation technology to commercial nurseries, encouraging farmers to adopt cultivated *T. indica* as a cash crop, and persuading pharmaceutical companies to source from sustainable *in-vitro* systems.

5. An Integrated Model: The Only Path Forward

No single strategy is a silver bullet. In-situ conservation is non-negotiable for preserving wild genetic diversity and ecological function, but it cannot meet market demand. Commercial cultivation using tissue-cultured plants can meet demand but carries risks of monoculture and genetic bottleneck if not managed with a diverse genetic base. *In-vitro* metabolite production is sustainable but requires significant capital investment. Therefore, the proposed Integrated Conservation and Utilisation Model (ICUM) is not just ideal but essential. Each component mitigates the weaknesses of the others. In-situ areas act as genetic reservoirs for biotechnological exploitation; cultivation provides raw material and economic benefit to local communities; and *in-vitro* production provides a high-tech, sustainable supply for industry, directly reducing the incentive to poach from the wild. Regulating the trade of wild-harvested material through CITES, while promoting certified cultivated and *in-vitro*-sourced products, is the necessary policy complement to this scientific framework.

Conclusion

Tylophora indica stands as a potent example of the global crisis facing medicinal plants. Its story underscores the unsustainable nature of relying solely on wild harvest for meeting modern pharmaceutical demand. This review conclusively demonstrates that the convergence of plant biotechnology and traditional conservation ethics offers a clear and viable path forward. Through the integrated application of high-throughput micropropagation for cultivation, advanced *in-vitro* culture systems for metabolite production, and cryotechniques for genetic safeguarding, the extinction of this invaluable species is preventable. The challenge is no longer scientific but rather one of political will, economic investment, and stakeholder collaboration. By embracing this multi-faceted approach, we can ensure that the therapeutic benefits of *Tylophora indica* are available for future generations while fulfilling our ethical obligation to preserve Earth's biodiversity.

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