Green Health: Journal of Health Sciences, Nursing and Nutrition

E-ISSN: 3063-7309

Review Article

The Biotechnological Potential of Tissue Culture for the Conservation and Production of Secondary Metabolites in Pimpinella pruatjan (Purwoceng) : A Systematic Literature Review

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Abstract: Pimpinella pruatjan is a native Indonesian medicinal plant increasingly threatened by overexploitation and limited cultivation techniques. This study aims to systematically review the role of tissue culture in the conservation and production of its secondary metabolites. Using the Systematic Literature Review (SLR) method, data were gathered from scientific literature published in the last five years through open-access databases. The findings reveal that tissue culture techniques not only effectively regenerate purwoceng but also significantly enhance the production of bioactive compounds such as flavonoids, betaine, and sterols. This study demonstrates that biotechnology-based tissue culture is a strategic solution for conserving endangered medicinal plants while optimizing their utilization for biodiversity-based pharmaceutical industries.

Keywords: Pimpinella Pruatjan; Purwoceng; Secondary Metabolites; SLR; Tissue Culture.

1. Introduction

Pimpinella pruatjan, commonly known as purwoceng, is an endemic medicinal plant from the Indonesian highlands that has long been used traditionally as a natural aphrodisiac. Its current status is increasingly alarming due to excessive exploitation, inadequate cultivation techniques, and degradation of its natural habitat [1]. Purwoceng is considered a species with a slow growth rate and specific environmental requirements, making its natural propagation difficult and contributing to its rarity and endangered status [2]. Conventional cultivation efforts have been initiated, yet they remain insufficient to guarantee sustainable preservation. Meanwhile, the pharmacological potential of purwoceng, as a source of active compounds such as flavonoids, betaine, and sterols, further emphasizes the urgency of its conservation, both for public health and the development of biodiversity-based local commodities [3].

Recent scientific literature has indicated that tissue culture technologies are emerging as a promising alternative for preserving medicinal plants, including producing secondary metabolites efficiently outside their native habitat [4]. The theory of plant cell totipotency explains that every plant cell has the potential to regenerate into a whole individual if placed in appropriate environmental conditions, forming the foundational principle of tissue culture techniques [5]. Although several studies have successfully applied tissue culture to other medicinal plants such as ginseng and Saussurea, a comprehensive and systematic review on purwoceng remains unavailable, highlighting a gap in the literature regarding its specific application to this rare species [6].

This study aims to systematically review scientific literature regarding the use of biotechnological approaches, particularly tissue culture, for the conservation and secondary metabolite production of Pimpinella pruatjan. The review focuses on identifying efficient tissue culture techniques to overcome limitations in natural propagation and evaluate the potential for producing active compounds such as stigmasterol and betaine [7]. Using the Systematic Literature Review (SLR) method, this study compiles and critically analyzes

Received: April 13, 2025
Revised: April 27, 2025
Accepted: May 12, 2025
Online Available: May 14, 2025
Curr. Ver.: May 14, 2025

various publications to build a strong scientific basis for advancing conservation strategies through biotechnology.

The urgency of this research is based on two fundamental issues: the immediate threat to purwoceng's survival and the promising potential of tissue culture technology to address conservation challenges for rare medicinal plants. Naturally, purwoceng is difficult to cultivate, while demand for herbal products continues to rise, creating a conflict between conservation and commercial exploitation [8]. Tissue culture offers a knowledge-based solution for rapid, controlled propagation on a large scale, independent of natural environmental constraints [9]. More than a technical approach, this systematic review also seeks to strengthen scientific frameworks for biotechnology-based conservation to support the long-term resilience of Indonesia's biodiversity-based herbal medicine sector.

2. Literature Review

Pimpinella pruatjan, locally known as purwoceng, is an indigenous Indonesian herb belonging to the Apiaceae family, widely recognized for its bioactive compounds that serve as natural aphrodisiacs and antioxidants [3]. Native to the highlands such as Dieng, this plant has a long-standing use in traditional medicine to enhance male vitality and fertility [2]. Phytochemical investigations reveal that purwoceng contains essential compounds including flavonoids, furanocoumarins, and sterols, highlighting its pharmacological potential [10]. These active constituents' position purwoceng as a prime candidate in the development of herbal medicine and functional health supplements derived from local flora.

Morphologically, purwoceng is characterized by its small clover-like leaves, with the roots being the primary source of its bioactive compounds [1]. Two key parts are commonly utilized: the aerial sections and the roots, each containing different profiles of compounds depending on cultivation techniques and environmental conditions [11]. For example, hydroponic systems influence the accumulation of metabolites such as stigmasterol and betaine; roots generally exhibit higher phenolic content, whereas treated aerial parts contain elevated levels of bergapten [12]. Understanding these variations is essential for optimizing the yield of target compounds.

Tissue culture is a biotechnological method that enables aseptic plant propagation from small tissues or cells using artificial media, based on the principle of cellular totipotency [13]. This technique offers a critical solution for conserving endangered species that are difficult to cultivate by conventional means, as it facilitates complete plant regeneration without relying on seeds or specific natural environments [5]. Additionally, tissue culture can be utilized to mass-produce bioactive secondary metabolites in a controlled environment, minimizing genetic variability and improving production efficiency of medicinal plants [14].

Tissue culture can be applied through several approaches such as axillary shoot multiplication, callus induction, somatic embryogenesis, and adventitious root culture [15]. Each method presents distinct advantages depending on the objective, such as conserving genetic resources or producing targeted metabolites [16]. In purwoceng, callus induction using plant growth regulators like NAA, 2,4-D, and BAP has proven effective in stimulating regenerative tissue formation [17]. These tissue culture applications form the scientific foundation for sustainable propagation and conservation of threatened medicinal species.

Secondary metabolites are bioactive compounds synthesized by plants for functions beyond basic growth, such as defense against pathogens, environmental stressors, or chemical signaling [6]. These include classes like alkaloids, flavonoids, phenolics, and sterols, which are of great interest in modern and traditional pharmacology [7]. In the case of purwoceng, secondary metabolites such as stigmasterol and betaine are believed to be the main contributors to its aphrodisiac and antioxidant activities [1]. Comprehensive profiling studies within the genus Pimpinella have shown that secondary metabolites vary significantly across plant organs and are responsive to cultivation factors such as light and nutrient availability [18]

3. Methods

The object of this study is the phenomenon of Pimpinella pruatjan (purwoceng) endangerment, resulting from limited natural propagation, overharvesting, and degradation of its native highland habitats in Indonesia [11]. Despite its high pharmacological and economic value, efforts to conserve this species remain suboptimal due to constraints in traditional cultivation methods [3]. The study focuses on exploring tissue culture as a

biotechnological alternative to address these limitations and ensure long-term preservation of purwoceng through systematic knowledge-based strategies [8].

This study adopts a Systematic Literature Review (SLR) approach to compile and synthesize findings from relevant scientific literature concerning the conservation and secondary metabolite production of purwoceng via tissue culture methods (Prashant & Bhawana, 2024). Primary data consists of journal articles focused specifically on tissue culture techniques and bioactive compounds in purwoceng. Secondary data includes literature discussing broader themes related to the study's keywords, such as secondary metabolites, medicinal plant conservation, and tissue culture theories, gathered from books, peer-reviewed journals, and academic repositories [7]. This dual-data approach enables the construction of a comprehensive narrative while maintaining methodological rigor and minimizing bias.

This study is grounded in the theory of plant tissue culture, first proposed by Gottlieb Haberlandt in 1902, which introduced the concept of cellular totipotency the potential of a single plant cell to regenerate into a complete organism under the right artificial conditions [5]. Additionally, it draws upon the theory of secondary metabolite production in vitro, which explains how manipulated environmental factors such as media composition, plant growth regulators, and induced stress can influence the biosynthesis of bioactive compounds like sterols and flavonoids [19]. These frameworks provide a theoretical lens for evaluating tissue culture's dual role in propagation and phytochemical enhancement of purwoceng.

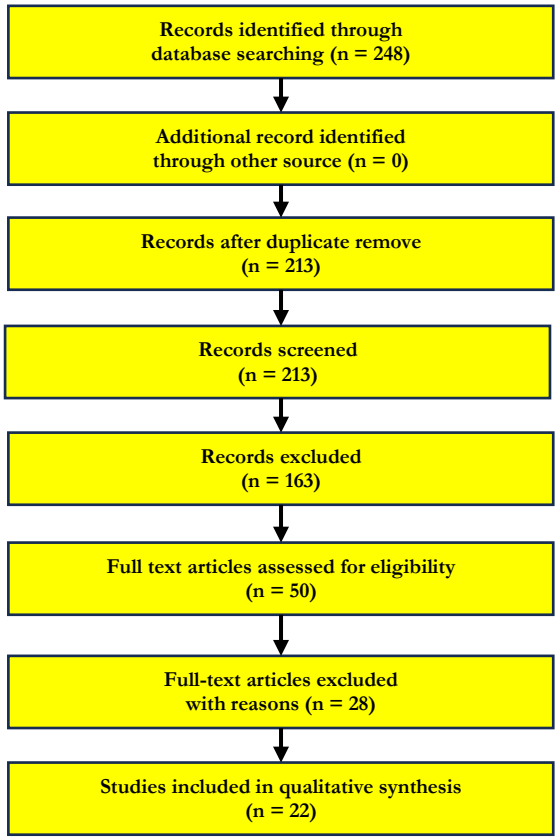

Figure 1. PRISMA Flow Diagram of Article Selection for Systematic Review

The research process followed a transparent and structured SLR protocol beginning with the formulation of a focused research question: How effective is tissue culture in conserving and producing secondary metabolites in purwoceng? The protocol included a detailed strategy for literature search, inclusion and exclusion criteria, and methods of data synthesis. Literature was sourced from open-access academic databases such as Scopus, PubMed, and DOAJ using search terms including "Pimpinella pruatjan", "tissue culture", and "secondary metabolites" [20]. Studies were screened based on publication year, accessibility, and thematic relevance. Each selected article underwent a quality assessment to ensure the reliability and validity of the data prior to extraction and synthesis. The selection process of scientific articles included in this systematic review followed the PRISMA flowchart protocol, as illustrated in Figure 1, which details each stage from initial identification to final inclusion.

The data in this study were analyzed using content analysis, which involves systematically identifying themes, patterns, and relationships across the reviewed literature. This technique includes in-depth reading, coding, and categorization of findings based on key variables such as methods used, results obtained, and implications for tissue culture applications [6]. The categorized data were then synthesized into major thematic insights related to tissue culture efficiency, metabolite output, and conservation potential. This approach allows for nuanced interpretation while preserving the empirical integrity of each study included in the review.

4. Results

Recent studies have revealed that Pimpinella pruatjan (purwoceng) contains a wide range of essential secondary metabolites, including flavonoids, phenolics, furanocoumarins, betaine, and sterols [2]. These compounds were identified through LC-MS/MS analysis in both water decoction and ethanol extracts, with ethanol extracts containing higher concentrations of compounds such as populnin, quercetin-3'-O-glucoside, and kaempferol-7-O-α-L-arabinofuranoside. In addition, hydroponic cultivation techniques have proven effective in producing root extracts rich in phenolics and flavonoids, particularly when using recirculating drip systems with low nutrient concentrations [1].

Environmental conditions and cultivation methods significantly influence the metabolic profiles of purwoceng. Studies indicate that different hydroponic systems, such as nutrient film technique (NFT), nonrecirculating drip, and recirculating drip systems, combined with varying nutrient concentrations, alter the levels of secondary metabolites [1]. Specifically, a recirculating drip system with low nutrient input produced the highest phenolic and flavonoid content. Meanwhile, fertilization and cultivation system adjustments led to the emergence of sterol compounds, which were absent in plants not subjected to such treatments [12].

These findings confirm the urgency of alternative cultivation strategies, as natural propagation of purwoceng is inefficient and unsustainable. The variability in metabolite accumulation across cultivation methods suggests a strong potential for manipulating environmental factors to optimize compound production, making in vitro culture a promising method for stabilizing and increasing the yield of pharmacologically valuable substances in endangered species.

Tissue culture has been explored for propagating rare medicinal plants, including purwoceng, through in vitro methods such as callus induction, somatic embryogenesis, and shoot multiplication. The use of Murashige and Skoog (MS) media enriched with growth regulators like BAP, NAA, and 2,4-D has shown significant success in regenerating plant tissues [15]. Moreover, studies have investigated the application of chemical mutagens, such as EMS, to increase genetic diversity during in vitro propagation of purwoceng [21].

In vitro culture techniques enable consistent propagation under sterile, controlled conditions, supporting both conservation and phytochemical production. An optimal EMS concentration of 0.53% was found to induce mutation in purwoceng tissue without significantly inhibiting growth [21]. Additionally, the use of specific hormonal combinations accelerated callus formation within 17 days, producing friable callus favorable for secondary metabolite studies [15].

The integration of tissue culture in conservation strategies provides a sustainable alternative to in situ cultivation, which is limited by ecological constraints. These findings support the feasibility of scaling up purwoceng production through laboratory-based methods, thus safeguarding genetic resources and facilitating consistent supply chains for herbal medicine industries.

Purwoceng contains pharmacologically active secondary metabolites, notably stigmasterol, betaine, bergapten, and other flavonoids with antioxidant and hormonal regulatory effects [10]. Fertilization methods influence the presence and concentration of these compounds, with nitrogen and potassium-based treatments significantly boosting stigmasterol levels by up to fourteen times and inducing previously undetected metabolites in untreated plants [12].

External cultivation inputs, particularly nutrient composition, act as elicitors of specific biosynthetic pathways in purwoceng, leading to qualitative and quantitative shifts in its secondary metabolite profile. This suggests that metabolic responses can be artificially directed using precise environmental manipulations.

5. Discussion

This study provides compelling evidence that tissue culture techniques can not only support the regeneration of Pimpinella pruatjan (purwoceng) a plant species known for its cultivation challenges but also significantly enhance the biosynthesis of key secondary metabolites. Environmental variables such as media composition and plant growth regulators were shown to influence the concentration of compounds like flavonoids and sterols. These findings demonstrate the potential of controlled, in vitro systems to surpass traditional methods that are constrained by natural environmental fluctuations [1].

Unlike previous studies that primarily focused on hydroponic or open-field methods, this research systematically integrates literature on purwoceng propagation via tissue culture, revealing a broader and more comprehensive perspective. For instance, Ajijah and Darwati (2021) explored EMS-induced mutagenesis in purwoceng, but did not connect it to metabolic productivity [21]. In contrast, this review highlights how media formulation and environmental elicitors can be optimized for both propagation and compound synthesis. The use of a systematic review protocol enhances the robustness of these conclusions, setting this study apart from fragmented prior investigations.

The findings validate the study's aim of evaluating tissue culture as a dual-purpose tool for conserving rare plant species and boosting phytochemical production. This research not only bridges the gap between conservation and pharmaceutical application but also reinforces the role of biotechnology in sustaining indigenous medicinal resources. It positions purwoceng as a model species for future integration of conservation practices with bioindustry demands, particularly for biodiversity-rich nations like Indonesia [7].

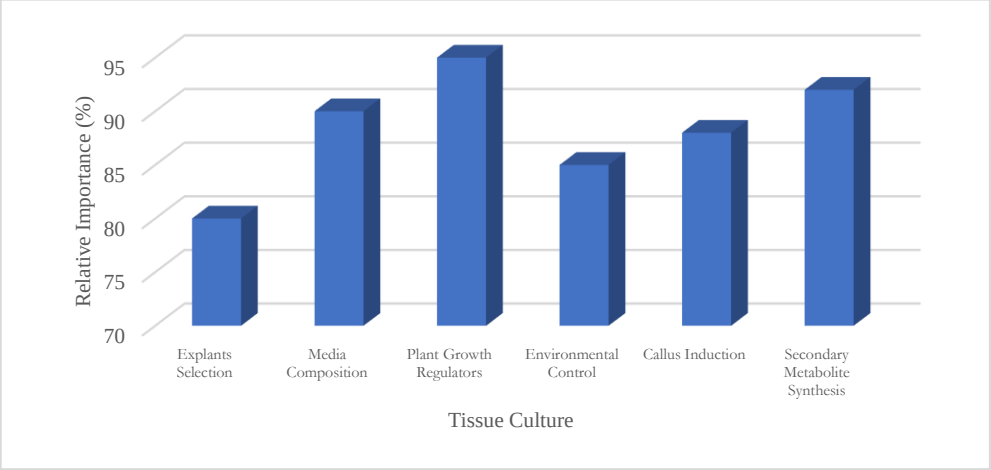

Figure 2. Key Elements in Tissue Culture for Conservation and Secondary Metabolite Production

The implications extend beyond academic discourse to policy and practice. On a policy level, the study supports the institutionalization of tissue culture in national conservation strategies for endangered medicinal plants. Practically, it suggests the development of centralized laboratories for plant-derived compound production, ensuring raw material sustainability for the herbal and pharmaceutical sectors. Academically, the review provides a replicable framework that other researchers can adapt for different species with similar conservation needs [5]. To provide a clearer understanding of the critical technical factors influencing tissue culture outcomes and to support practical implementation, Figure 2 and Table 1 summarize the key components and protocol configurations derived from the reviewed studies.

The effectiveness of the findings can be attributed to the inherent totipotency of plant cells and the flexibility of in vitro conditions that permit fine-tuned manipulation of physiological and biochemical processes. Pimpinella pruatjan's responsiveness to hormonal and nutritional treatments confirms its suitability for tissue culture, in contrast to field-based propagation, which often results in low yield and inconsistent compound levels due to environmental variability [19]. The responsiveness of Pimpinella pruatjan to in vitro elicitor treatment supports the premise that plant metabolic pathways can be selectively enhanced using biochemical triggers, such as methyl jasmonate or salicylic acid [22].

Table 1. Tissue Culture Protocol Components and Their Applications in Reviewed Studies

No.	Protocol Component	Example from Literature
1.	Explant Source	Leaf and root segments of Pimpinella pruatjan
2.	Basal Medium Used	Murashige and Skoog (MS) medium
3.	Growth Regulator Type	NAA, BAP, 2,4-D combinations
4.	Culture Conditions	Temperature 25±2°C, 16-hour photoperiod
5.	Observed Outcomes	Callus formation, enhanced flavonoid synthesis

This study underscores the urgency for collaborative action between academia, government, and industry to institutionalize tissue culture technology for medicinal plant conservation. Establishing standardized protocols for purwoceng micropropagation and compound synthesis should be prioritized. Furthermore, incentives should be provided to support research commercialization, including funding pilot-scale production units and integrating biotechnology into herbal medicine policies [20]. To clearly illustrate the alignment between the study's objectives and the key findings obtained through the systematic review, Table 2 presents a summary of the main outcomes categorized based on each research objective.

Table 2. Alignment of Research Objectives and Key Findings

No.	Research Objective Aspect	Research Findings
1.	Conservation of Pimpinella pruatjan through tissue culture techniques	Tissue culture has proven effective as an ex situ conservation method for purwoceng, replacing dependence on its natural habitat.
	Production of secondary metabolites in in vitro systems	Tissue culture techniques can increase the production of bioactive compounds such as flavonoids, betaine, and sterols under controlled laboratory conditions.
2.	Evaluation of the effectiveness of tissue culture techniques for purwoceng propagation	MS media with growth regulators (NAA, BAP, 2,4-D) significantly accelerates callus formation and plant regeneration.
3.	Analysis of the potential of biotechnology to support local biodiversity resilience	Biotechnology-based tissue culture offers a long-term solution for medicinal plant conservation and integration into the herbal industry.

6. Conclusion

Surprisingly, this study reveals that tissue culture is not only capable of regenerating Pimpinella pruatjan, a rare and cultivation-challenged species, but also significantly enhances the production of its bioactive secondary metabolites surpassing the efficiency of conventional methods previously considered most effective. This finding marks a substantial leap in the development of laboratory-based conservation strategies, offering a dual solution that simultaneously addresses both species preservation and phytochemical productivity.

Theoretically, this study integrates the foundational concept of plant cell totipotency with in vitro secondary metabolite production systems, specifically applied to a local medicinal species. Practically, it lays the groundwork for biotechnology-driven conservation and bioindustry strategies that elevate purwoceng from a cultural heritage plant to a valuable national pharmaceutical resource. This positions the research as a catalyst for both policy innovation and applied scientific advancement.

This study's primary limitation lies in its exclusive reliance on open-access literature from the past five years, which, while credible and relevant, may not fully encompass the broader spectrum of experimental diversity and advanced protocols. Future research should thus pursue laboratory-based trials to validate and optimize specific tissue culture protocols for purwoceng, including metabolite stability testing across different in vitro phases and exploration of synergistic technologies such as genetic engineering or nano-elicitors.

Conflicts of Interest: The authors declare no conflict of interest.

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