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In vitro propagation and phytochemical screening in *Coleus vettiveroides* K.C. Jacob

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Abstract
Coleus vettiveroides K.C. Jacob, a medicinal plant belonging to the family Lamiaceae, has gained considerable scientific attention due to its rich phytochemical profile and therapeutic potential. This manuscript presents a comprehensive review of in vitro propagation techniques and phytochemical screening methodologies applicable to *C. vettiveroides*, drawing from protocols established in closely related *Coleus* and *Plectranthus* species. Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) has been identified as effective for shoot multiplication and rooting. Phytochemical screening reveals the consistent presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and steroids in *Coleus* species, all of which contribute to recognized medicinal properties. In vitro culture systems offer a sustainable platform for mass propagation and enhanced secondary metabolite production, directly addressing conservation concerns and growing pharmaceutical demands. This review synthesizes current knowledge and provides a scientific foundation for future research on *C. vettiveroides* biotechnology, conservation, and drug development.

Keywords: *Coleus vettiveroides*, in vitro propagation, micropropagation, phytochemical screening, secondary metabolites, tissue culture, plant growth regulators

Introduction

Medicinal plants have served as the foundation of traditional healthcare systems globally, supplying bioactive compounds essential to pharmaceutical discovery and development. The genus *Coleus*, belonging to the Lamiaceae family, encompasses approximately 350 species distributed across tropical and subtropical regions of Africa and Asia (Greyvenstein, 2009) [8]. Among these, *Coleus vettiveroides* K.C. Jacob is a particularly valuable aromatic species with documented ethnomedicinal uses and a diverse secondary metabolite profile (Saraswathy *et al*., 2011) [16]. Growing pharmaceutical demand, progressive habitat loss, and unsustainable harvesting practices have collectively raised conservation concerns for this species (Sarasan *et al*., 2011) [17].

In vitro propagation techniques provide a scientifically validated and sustainable solution for mass multiplication of medicinal plants. These methods maintain genetic uniformity, allow year-round production independent of seasonal and climatic constraints, and support conservation initiatives for endangered or over-exploited species (Thangavel *et al*., 2014; Chatterjee & Ghosh, 2020) [3, 25]. Beyond mere multiplication, tissue culture also serves as a powerful platform for the controlled production of plant secondary metabolites, enabling manipulation of biosynthetic pathways under defined conditions (Espinosa-Leal et al., 2018; Gonçalves & Romano, 2018) [6, 7]. These capabilities have attracted increasing scientific attention in recent decades, with biotechnological tools now regularly deployed for medicinal plant conservation and pharmaceutical compound production (Singh *et al*., 2017; Sharma, 2014) [18, 20].

Coleus vettiveroides has been utilized in traditional medicine across South and Southeast Asia for the management of various ailments, and its phytochemical richness supports its therapeutic reputation (Saraswathy *et al*., 2011) [16]. Despite its recognized importance, standardized *in vitro* propagation protocols and comprehensive phytochemical profiling for this species remain limited in the published literature. This knowledge gap restricts both conservation efforts and the exploration of its pharmaceutical potential. The present manuscript aims to bridge this gap by reviewing applicable micropropagation strategies drawn from related Lamiaceae members, discussing phytochemical screening approaches, and exploring avenues for tissue culture-based secondary metabolite production in *C. vettiveroides*.

Botanical and Pharmacognostic Background

Coleus vettiveroides K.C. Jacob is an aromatic perennial herb belonging to the Lamiaceae family. The plant is characterized by its distinctive odor, arising from the secretion of volatile terpenoids and aromatic compounds from glandular trichomes on its surface (Saraswathy *et al*., 2011) [16]. The genus *Coleus* has undergone significant taxonomic revision in recent decades, with several species now reclassified under *Plectranthus*; however, *C. vettiveroides* has retained its original designation (Greyvenstein, 2009) [8]. The species is distributed in specific ecological zones of South India, where it has been used in traditional Ayurvedic formulations for centuries (Saraswathy *et al*., 2011) [16].

The pharmacognostic evaluation of *C. vettiveroides* has documented its macromorphological and micromorphological characteristics, essential oil composition, and preliminary phytochemical profile (Saraswathy *et al*., 2011) [16]. These pharmacognostic standards are essential for quality control and authentication of raw materials used in herbal medicine preparation. The Lamiaceae family is broadly renowned for producing a wide spectrum of secondary metabolites, including monoterpenes, sesquiterpenes, diterpenes, phenolic acids, and flavonoids, all of which contribute to the therapeutic properties of member species (Cho *et al*., 2019; Tarh *et al*., 2015) [4, 24]. Numerous *Coleus* species have been investigated for antibacterial, antifungal, antioxidant, anti-inflammatory, and

cytotoxic activities, and *C. vettiveroides* shares many of these phytochemical classes (Tarh *et al*., 2015; Saraswathy *et al*., 2011) [16, 24].

The conservation status of *C. vettiveroides* is a matter of growing concern. Overexploitation for use in traditional medicine, combined with limited cultivation efforts, has reduced wild populations in several regions (Sarasan *et al*., 2011) [17]. This underscores the urgency of developing reliable in vitro propagation protocols to supplement natural populations and satisfy industrial demand without depleting wild resources.

In Vitro Propagation Techniques

1. Explant Selection and Surface Sterilization

The success of any *in vitro* propagation protocol is fundamentally dependent on the selection of an appropriate explant type and an effective surface sterilization procedure. For *Coleus* and related Lamiaceae members, various explant types have been successfully employed, including nodal segments, shoot tips, and leaf disc tissues (Greyvenstein, 2009; Cho *et al*., 2019; Laslo et al., 2011) [4, 8, 11]. Among these, nodal segments are considered the most reliable, as they contain preexisting axillary meristems that facilitate direct organogenesis, thereby minimizing the risk of somaclonal variation commonly associated with callus-mediated regeneration pathways (Sinha, 2015; Sreedevi & Damodharam, 2015) [21, 23].

Standard sterilization protocols for Lamiaceae explants typically involve a multi-step procedure: initial washing of explants with running tap water and a mild detergent, immersion in 70% ethanol for 30–60 seconds, followed by treatment with sodium hypochlorite solution (0.5–1.05%) containing 0.1% Tween-20 surfactant for 10–20 minutes, and finally three to five washes with sterile distilled water (Greyvenstein, 2009; Laslo *et al*., 2011) [8, 11]. For *Coleus blumei*, 0.5% Chloruss solution for 8 minutes combined with 0.1% mercuric chloride for 5 minutes yielded satisfactory contamination-free cultures (Laslo *et al*., 2011) [11]. The duration of exposure to sterilizing agents must be optimized for each species and tissue type to balance elimination of microbial contaminants against maintenance of explant viability (Pan *et al*., 2013; Sreedevi & Damodharam, 2015) [14, 23].

2. Culture Media and Plant Growth Regulators

Murashige and Skoog (MS) medium has been established as the most widely used and effective basal medium for *in vitro* propagation of Lamiaceae medicinal plants, including *Coleus* species (Greyvenstein, 2009; Cho *et al*., 2019; Sinha, 2015) [4, 8, 21]. The standard formulation contains macro and microelements, vitamins (thiamine, pyridoxine, nicotinic acid), myo-inositol, 3% sucrose as the primary carbon source, and 0.7–0.8% agar as a gelling agent, with pH adjusted to 5.7–5.8 before autoclaving at 121°C for 15–20 minutes (Cho *et al*., 2019; Laslo *et al*., 2011) [4, 11]. Half-strength MS formulations are commonly employed for rooting phases and for sensitive explants that may respond adversely to full-strength ionic concentrations (Greyvenstein, 2009) [8].

Plant growth regulators (PGRs) are fundamental to directing morphogenic responses in tissue culture. The cytokinin-to-auxin ratio largely determines whether cultures undergo shoot organogenesis, root initiation, or callus proliferation. Cytokinins — principally BAP, kinetin, and zeatin — are employed at varying concentrations to stimulate axillary bud break and multiple shoot induction (Sinha, 2015; Singh *et al*., 2017; Laslo *et al*., 2011) [11, 20, 21]. Auxins such as NAA, indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) at low concentrations (0.1–1.5 mg/L) are used to promote adventitious root development and, at higher concentrations, callus formation (Sinha, 2015; Pan *et al*., 2013) [14, 21]. The optimal PGR type and concentration combination varies among species and must be empirically determined through systematic experimentation.

3. Callus Induction

Callus culture is an important intermediate in tissue culture workflows, providing undifferentiated cell masses that serve as starting material for cell suspension cultures, somatic embryogenesis, and secondary metabolite production (Reddy *et al*., 2013; Kajiki, 1996; Bakshi, 2009) [1, 9, 15]. For *Coleus* and *Plectranthus* species, callus induction has been most effectively achieved using the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with low concentrations of cytokinins (Greyvenstein, 2009; Kajiki, 1996) [8, 9]. In ornamental *Plectranthus*, friable callus was produced using 2,4-D (0.1–1.0 mg/L) combined with BAP (0.1–0.5 mg/L) or kinetin (0.1–1.0 mg/L) on MS medium (Greyvenstein, 2009) [8].

Light conditions significantly modulate callus induction efficiency and morphology. Dark culture conditions generally favor callus initiation in many Lamiaceae genotypes, while continuous light may suppress or alter callus texture (Greyvenstein, 2009) [8]. The texture of callus — whether compact or friable — determines its suitability for cell suspension culture. Friable, light-colored callus is strongly preferred for establishing suspension systems destined for metabolite extraction. For *Pfaffia glomerata*, optimal callus formation and secondary metabolite accumulation was achieved on medium supplemented with 1.0 mg/L 2,4-D and 0.5 mg/L kinetin (Kajiki, 1996) [9], a protocol that serves as a useful reference for *C. vettiveroides* callus optimization studies.

4. Shoot Proliferation and Multiplication

Multiple shoot proliferation is the central phase of any micropropagation protocol, directly determining the efficiency and commercial viability of the propagation system. In *Coleus* species, cytokinins — especially BAP — are indispensable for initiating and sustaining axillary shoot multiplication (Greyvenstein, 2009; Laslo *et al*., 2011) [8, 11]. A key finding from ornamental *Plectranthus* studies is that relatively low BAP concentrations (0.1 mg/L) yielded the highest shoot numbers, indicating that these species are sensitive to cytokinin excess and that high concentrations may be inhibitory (Greyvenstein, 2009) [8]. This finding is directly relevant to *C. vettiveroides* protocol design, as it argues for beginning optimization at low cytokinin levels rather than high ones.

In *Coleus blumei*, multiple shoot induction was achieved with BAP, kinetin, and 2-isopentenyl adenine (2iP) at concentrations of 0.5–1.5 mg/L, with cytokinin type and concentration significantly influencing both the number and quality of regenerated shoots (Laslo *et al*., 2011) [11]. Standard subculture intervals of 4–6 weeks on multiplication medium ensure continued vigorous growth and prevent phenolic browning of explants. The presence of auxins during the shoot multiplication phase is generally not

required and, in several Lamiaceae species, has been shown to reduce shoot proliferation rates (Greyvenstein, 2009) [8]. Adding activated charcoal (0.1–0.5%) to the medium may be beneficial to adsorb inhibitory phenolic compounds released by wounded explants.

5. Rooting and Acclimatization

The rooting phase completes the micropropagation cycle and produces plantlets capable of surviving transfer to soil. Rooting in *Coleus* species can be achieved either *in vitro* on auxin-supplemented or hormone-free medium, or ex vitro through direct insertion of shoots into rooting substrates (Greyvenstein, 2009; Laslo *et al*., 2011) [8, 11]. In *Plectranthus* species, rooting occurred spontaneously on hormone-free half-strength MS medium within 2–3 weeks, reflecting the inherent ease of rooting in these genera (Greyvenstein, 2009) [8]. For *Coleus blumei*, NAA or IAA at 0.5–1.5 mg/L on half-strength MS enhanced both rooting percentage and root system quality (Laslo *et al*., 2011) [11].

Ex vitro rooting provides economic and practical advantages. In *Plectranthus* accessions, near 100% rooting was achieved by dipping shoot bases in 500 ppm IBA solution before inserting into a peat:perlite (1:1) substrate under high-humidity conditions (Greyvenstein, 2009) [8]. This approach eliminates an additional sterile culture phase and reduces production costs. Acclimatization of *in vitro*-rooted plantlets to ex vitro conditions is a critical transition step. Plantlets are first exposed to ambient humidity gradually — beginning with loosened vessel caps, then transfer to polythene-covered trays — before eventual placement in greenhouse conditions (Greyvenstein, 2009; Sinha, 2015) [8, 21]. *Tinospora cordifolia* plantlets were successfully acclimatized in sterile garden soil:sand (3:1) and treated with fungicide solution prior to field transfer (Sinha, 2015) [21]. Substrate mixtures enriched with vermicompost have also demonstrated excellent establishment rates in Lamiaceae plantlets (Sreedevi & Damodharam, 2015) [23].

Phytochemical Screening

1. Secondary Metabolites in Medicinal Plants

Plant secondary metabolites are structurally diverse organic compounds not directly involved in fundamental growth or reproductive processes, yet they play essential roles in chemical defense, pollinator attraction, and environmental adaptation (Espinosa-Leal *et al*., 2018; Gonçalves & Romano, 2018) [6, 7]. They represent one of the richest natural libraries of bioactive molecules available for pharmaceutical, nutraceutical, and agrochemical development (Mulabagal & Tsay, 2004; de Souza *et al*., 2018) [5, 12]. The main structural classes — alkaloids, terpenoids, phenolics, and glycosides — differ in their biosynthetic origins, physicochemical properties, and biological activities.

Members of the Lamiaceae family are particularly prolific producers of terpenoids (monoterpenes, diterpenes, and sesquiterpenes) and phenolic compounds (caffeic acid derivatives, flavonoids, and rosmarinic acid), which underlie their extensive use in traditional and modern medicine (Greyvenstein, 2009; Tarh et al., 2015) [8, 24]. Secondary metabolite accumulation is regulated by a complex interplay of developmental signals, environmental stressors, and epigenetic factors (Gonçalves & Romano, 2018; Mulabagal & Tsay, 2004) [7, 12]. Plant tissue culture systems permit experimental manipulation of these regulators, enabling strategic enhancement of target compound production under fully controlled laboratory conditions (Espinosa-Leal *et al*., 2018; Skrzypczak-Pietraszek *et al*., 2018) [6, 22].

2. Phytochemical Tests and Analysis

Preliminary phytochemical screening involves systematic qualitative testing of plant extracts for the presence of major classes of secondary metabolites using simple wet chemistry methods (Sinha, 2015; Sreedevi & Damodharam, 2015) [21, 23]. Alkaloids are detected using Mayer's reagent (cream precipitate), Wagner's reagent (reddish-brown precipitate), and Dragendorff's reagent (orange-red precipitate). Flavonoids are identified through color reactions with aluminum chloride (yellow fluorescence), sodium hydroxide (intense yellow), or the Shinoda test using magnesium ribbon and concentrated HCl (pink to red coloration). Tannins are revealed by treatment with 1% ferric chloride, producing blue-black coloration for hydrolysable tannins and greenish-black for condensed tannins (Sinha, 2015; Sreedevi & Damodharam, 2015) [21, 23].

Saponins are detected by vigorous agitation of aqueous extracts, which produces stable persistent foam due to their amphipathic surfactant nature. Terpenoids are identified using the Salkowski reaction (reddish-brown interface layer in chloroform:concentrated H_2SO_4). Steroids are distinguished from terpenoids using the Liebermann-Burchard test (green to blue coloration). Phenolic compounds are quantified using Folin-Ciocalteu reagent, producing a blue chromophore proportional to total phenol content (Sreedevi & Damodharam, 2015; Cabañas García *et al*., 2019) [2, 23].

For advanced characterization, High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) provide precise identification and quantification of individual compounds and are essential for differentiating structurally similar molecules within the same phytochemical class (Kajiki, 1996; Skrzypczak-Pietraszek *et al*., 2018) [9, 22]. UHPLC-MS is increasingly applied for comprehensive metabolomics profiling in both field-grown and tissue culture-derived plant material (Cabañas García *et al*., 2019) [2].

3. Bioactive Compounds in *Coleus* Species

Phytochemical investigations of *Coleus* species have consistently demonstrated the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and steroids (Tarh *et al*., 2015; Saraswathy *et al*., 2011) [16, 24]. These compounds collectively account for the broad spectrum of biological activities reported for genus members, including antibacterial, antioxidant, anti-inflammatory, and cytotoxic effects. The antibacterial activity observed in *Coleus* extracts has been attributed principally to phenolic compounds and terpenoids, which disrupt bacterial membrane integrity and interfere with metabolic enzyme systems (Tarh *et al*., 2015) [24]. Flavonoids present in these extracts contribute significantly to antioxidant activity through free-radical scavenging, metal ion chelation, and inhibition of lipid peroxidation (Sreedevi & Damodharam, 2015) [23].

Coleus vettiveroides specifically has been documented for its characteristic essential oil composition — dominated by sesquiterpene hydrocarbons and oxygenated terpenoids — alongside phenolic acids and flavonoid glycosides in its

non-volatile fraction (Saraswathy *et al*., 2011) [16]. These phytochemical classes align closely with those reported for *Plectranthus* species, reinforcing the relevance of cross-species comparisons for guiding research protocols (Greyvenstein, 2009) [8]. Saponins and alkaloids, though typically minor constituents in Lamiaceae members, have also been detected in *Coleus* extracts and may contribute to observed bioactivities. Bioassay-guided fractionation of *C. vettiveroides* extracts is strongly recommended to isolate and characterize the specific molecules responsible for its traditional therapeutic applications.

Secondary Metabolite Production through Tissue Culture

Plant tissue culture systems confer several strategic advantages for secondary metabolite production, including independence from seasonal and geographic constraints, ability to maintain defined growth conditions, and the capacity to select and scale up high-producing cell lines (Espinosa-Leal *et al*., 2018; Gonçalves & Romano, 2018; Mulabagal & Tsay, 2004) [6, 7, 12]. Cell suspension cultures derived from friable callus are particularly suitable for large-scale bioreactor-based production, enabling continuous or semi-continuous harvest of metabolites (Orozco Sánchez *et al*., 2002; Krishnan *et al*., 2020) [10, 13].

The accumulation of secondary metabolites in tissue cultures is profoundly influenced by PGR balance, carbon source availability, light regime, temperature, and the presence of elicitors or precursors. High auxin:cytokinin ratios typically favor biomass expansion over secondary metabolite synthesis, while specific stress stimuli — such as UV irradiation, heavy metals, or fungal elicitors — can trigger dramatic increases in metabolite output (Kajiki, 1996; Singh *et al*., 2017) [9, 20]. In *Charybdis congesta*, callus cultures successfully produced bufadienolides including proscillaridin A and scillaren A at physiologically relevant concentrations (Reddy *et al*., 2013) [15]. In *Vitex agnus castus* shoot cultures, supplementation with cinnamic acid and phenylalanine as biosynthetic precursors significantly amplified the yield of polyphenolic compounds (Skrzypczak-Pietraszek *et al*., 2018) [22]. Similarly, *Morus alba* tissue culture systems were successfully optimized for phytoestrogen enhancement using medium supplementation strategies (Bakshi, 2009) [1]. These examples provide clear precedent and strategic guidance for developing *C. vettiveroides* metabolite production platforms.

Discussion and Conclusion

The integration of evidence from *Plectranthus*, *Coleus blumei*, and related Lamiaceae species yields a well-grounded preliminary framework for *in vitro* propagation of *C. vettiveroides*. MS medium supplemented with low BAP concentrations (0.1–0.5 mg/L) and NAA (0.5–1.0 mg/L) represents a biologically plausible and experimentally supported starting point for shoot multiplication and *in vitro* rooting protocols (Greyvenstein, 2009; Laslo et al., 2011) [8, 11]. Nodal segments, obtained from actively growing axenic stock plants, are the preferred explant type on account of their meristematic activity and genetic stability (Sinha, 2015) [21]. Systematic evaluation of additional explant types (leaf discs, shoot tips), cytokinin alternatives (zeatin, 2iP), and auxin combinations will be necessary to define the optimal regeneration pathway for this species.

Callus induction using 2,4-D combined with BAP or kinetin on MS medium will establish the foundation for cell suspension culture development, thereby enabling large-scale secondary metabolite production in bioreactor systems (Greyvenstein, 2009; Kajiki, 1996; Orozco Sánchez *et al*., 2002) [8, 9, 13]. Comprehensive phytochemical profiling of both field-grown and *in vitro*-propagated *C. vettiveroides* material — using parallel qualitative screening and HPLC/GC-MS analysis — will clarify which metabolite classes are maintained or enriched in culture conditions (Cabañas García *et al*., 2019; Sarasan *et al*., 2011) [2, 17]. This comparative data is critical for validating the pharmaceutical equivalence of tissue culture-derived plant material.

Future research priorities for *C. vettiveroides* biotechnology should encompass molecular characterization of key secondary metabolite biosynthetic genes, transcriptomic and metabolomic analyses across developmental stages and culture conditions, and evaluation of elicitor and precursor feeding strategies to maximize bioactive compound yields (Singh *et al*., 2017; Gonçalves & Romano, 2018) [7, 20]. *In vitro* conservation strategies — slow-growth maintenance and cryopreservation — should be implemented in parallel to establish long-term germplasm banks that safeguard the genetic diversity of wild *C. vettiveroides* populations (Thangavel *et al*., 2014; Singh, 2019) [19, 25].

In conclusion, *Coleus vettiveroides* K.C. Jacob is a medicinally important species whose full biotechnological potential awaits systematic exploration. The integration of *in vitro* propagation with rigorous phytochemical screening provides a holistic and scientifically robust strategy for conservation, sustainable utilization, and pharmaceutical development of this species. Protocols adapted from closely related Lamiaceae members provide a scientifically grounded starting point, with optimization studies and advanced analytical characterization forming the logical next steps. Enhanced secondary metabolite production through tissue culture will ultimately support the evidence-based transition of *C. vettiveroides* bioactive compounds from ethnomedicinal tradition to modern therapeutic application.

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