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In vitro propagation and phytochemical screening in *coleus amboinicus*

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Abstract
Coleus amboinicus (Lour.) (syn. *Plectranthus amboinicus*), commonly known as Indian borage or Mexican mint, is a therapeutically important aromatic medicinal plant belonging to the family Lamiaceae. This manuscript comprehensively reviews the *in vitro* propagation techniques and phytochemical profiles of *C. amboinicus*, emphasizing its potential for sustainable production and pharmaceutical applications. Micropropagation protocols utilizing Murashige and Skoog (MS) medium supplemented with various concentrations of plant growth regulators including 6-benzylaminopurine (BAP), naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) have been successfully established for explant sterilization, callus induction, shoot proliferation, and root development. Optimal shoot multiplication has been achieved using MS medium with 0.4 mg/L BAP, while rooting is best accomplished on half-strength MS medium. Phytochemical screening reveals the presence of diverse bioactive compounds including alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, and essential oils rich in carvacrol and thymol. These phytochemicals exhibit significant antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities against various pathogens and disease models. Tissue culture-derived plants maintain comparable essential oil profiles to field-grown specimens, demonstrating the viability of *in vitro* propagation for commercial production. This review synthesizes current knowledge on micropropagation strategies and phytochemical characterization, highlighting the importance of *C. amboinicus* as a valuable medicinal plant resource and the role of biotechnological approaches in its conservation and sustainable utilization.

Keywords: *Coleus amboinicus,* Plectranthus amboinicus, micropropagation, tissue culture, phytochemical screening, essential oils, carvacrol, thymol, plant growth regulator

Introduction

Coleus amboinicus (Lour.), also known as *Plectranthus amboinicus* (Lour.) Sprengel, is a perennial aromatic herb belonging to the family Lamiaceae that has been extensively utilized in traditional medicine systems across Asia, Africa, and South America (Arumugam *et al*., 2020 Silva *et al*., 2020) [4, 40]. This succulent plant, commonly referred to as Indian borage, Mexican mint, Cuban oregano, or country borage, is characterized by its thick, fleshy leaves with a distinctive thyme-like aroma and has been traditionally employed for treating various ailments including bronchitis, asthma, diarrhea, epilepsy, renal calculi, and fever (Sharma *et al*., 2015; Gurgel *et al*., 2009) [17, 38]. The therapeutic potential of *C. amboinicus* has attracted considerable attention from pharmaceutical and nutraceutical industries, leading to increased demand and subsequent overexploitation of wild populations (Arumugam *et al*., 2020 Sarasan *et al*., 2011) [4, 37].

The uncontrolled harvesting of *C. amboinicus* from natural habitats has raised concerns about the sustainability of this valuable medicinal plant resource and the risk of genetic erosion (Sarasan *et al*., 2011; Afolayan & Adebola, 2004) [3, 37]. Conventional propagation methods through stem cuttings, while feasible, are often insufficient to meet the growing commercial demand and may not ensure genetic uniformity or year-round availability (Belniaki *et al*., 2018) [7]. Consequently, there is an urgent need for alternative propagation strategies that can facilitate rapid multiplication, ensure genetic stability, and support conservation efforts while meeting industrial demands.

In vitro propagation, commonly referred to as micropropagation or plant tissue culture, represents a powerful biotechnological tool for the rapid, large-scale, and year-round multiplication of medicinally important plants (Afolayan & Adebola, 2004 [3] Pant, 2014). Tissue culture techniques enable the regeneration of complete plants from various explant sources under controlled aseptic conditions, maintaining genetic fidelity of the parent material (Opabode & Akinyemiju, 2016) [28]. The technique has been successfully applied to numerous medicinal plants for conservation, commercial production, and secondary metabolite enhancement purposes (Grevenstuk & Romano, 2013 Sarasan *et al*., 2011) [12, 37].

Phytochemical screening is an essential preliminary step in the characterization of medicinal plants, facilitating the identification and quantification of bioactive compounds responsible for therapeutic activities (Paul *et al*., 2014; Nagalakshmi *et al*., 2012) [27, 30]. *C. amboinicus* is known to be a rich source of diverse phytochemicals including alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, and volatile essential oils (Bhatt *et al*., 2013 [8] Gupta *et al*., 2013 Laungsuwon, 1997) [13, 26]. The essential oil of *C. amboinicus* is primarily composed of carvacrol and thymol, monoterpenes known for their potent antimicrobial and antioxidant activities (Velasco *et al*., 2009; Roja *et al*., 2006) [34, 49].

The biological activities attributed to *C. amboinicus* extracts and essential oils are extensive and include antimicrobial, antioxidant, anti-inflammatory, antifungal, anthelmintic, and cytotoxic properties (Bhattacharjee *et al*., 2010 [9]

Vasconcelos *et al*., 2017; Khattak & Taher, 2011) [20, 48]. These activities have been validated through numerous *in vitro* and *in vivo* studies, providing scientific substantiation for the plant's traditional medicinal uses. The integration of micropropagation with phytochemical analysis offers a comprehensive approach to the sustainable utilization of this valuable plant resource.

Despite the considerable interest in *C. amboinicus*, there remains a need for a comprehensive synthesis of the available knowledge on its *in vitro* propagation protocols and phytochemical profiles. This manuscript aims to compile and critically analyze the existing literature on micropropagation techniques and phytochemical screening of *C. amboinicus*, discussing the optimization of tissue culture parameters, the chemical diversity of phytochemical constituents, and the pharmacological significance of identified bioactive compounds. The review ultimately seeks to provide a scientific foundation for the sustainable development of *C. amboinicus* as a commercially viable medicinal plant.

Materials and Methods

The methods described in this review are synthesized from published literature on *in vitro* propagation and phytochemical screening of *Coleus amboinicus*. The protocols represent optimized procedures derived from multiple studies, with specific conditions noted where significant variation exists across reports.

1. Plant Material and Explant Selection

Nodal segments and shoot tip explants from actively growing vegetative shoots of *C. amboinicus* are the most commonly used explant types for micropropagation due to their high regenerative capacity and ease of surface sterilization (Ab Rahman *et al*., 2015 [1] Arumugam *et al*., 2020) [4]. Nodal segments of approximately 1.0-2.0 cm length, each containing one or two axillary buds, are cut from healthy, disease-free donor plants maintained under greenhouse conditions (Ab Rahman *et al*., 2015) [1]. Leaf explants and stem segments have also been employed for callus induction, though with variable success rates depending on plant growth regulator combinations (Roja *et al*., 2006 Simanjuntak *et al*., 2017) [34, 41]. Young, fully expanded leaves from the fourth to sixth node of actively growing shoots are preferred for leaf-based callus induction protocols (Roja *et al*., 2006) [34]. Donor plant selection and maintenance significantly influence explant quality and subsequent *in vitro* response, with plants maintained under optimal nutritional and environmental conditions providing superior explants (Rajasekharan, 2010) [32].

2. Surface Sterilization

Surface sterilization is a critical step in establishing aseptic tissue cultures and typically follows a standardized protocol with some variation across laboratories. Explants are first washed under running tap water for 15-30 minutes to remove surface contaminants, followed by rinsing with distilled water (Ab Rahman *et al*., 2015) [1]. Under laminar flow conditions, explants are treated with 70% ethanol for 30-60 seconds, followed by surface sterilization with 0.1-0.2% (w/v) mercuric chloride (HgCl2) for 5-10 minutes or 20-30% commercial bleach (sodium hypochlorite) for 10-20 minutes (Ab Rahman *et al*., 2015 [1] Arumugam *et al*., 2020) [4]. The sterilization treatment is followed by 3-5 rinses with sterile distilled water to remove residual sterilizing agents (Ab Rahman *et al*., 2015) [1]. Explant ends are trimmed by 2-3 mm to remove chemically damaged tissue before inoculation onto culture medium (Arumugam *et al*., 2020) [4]. The optimal sterilization protocol balances surface sterility with explant viability, as excessively harsh treatment leads to tissue browning and reduced regeneration frequency (Dube *et al*., 2011) [11].

3. Culture Media and Growth Regulators

Murashige and Skoog (MS) medium is the most widely used basal medium for *in vitro* propagation of *C. amboinicus* due to its balanced composition of macro- and micronutrients (Ab Rahman *et al*., 2015 [1] Arumugam *et al*., 2020 [4] Dube *et al*., 2011) [11]. The medium is supplemented with 3% (w/v) sucrose as a carbon source and solidified with 0.8% (w/v) agar (Ab Rahman *et al*., 2015) [1]. The pH of the medium is adjusted to 5.7-5.8 before autoclaving at 121 degrees C and 15 psi for 15-20 minutes (Ab Rahman *et al*., 2015 Rajasekharan, 2010) [1, 32].

Plant growth regulators play a crucial role in regulating morphogenesis in tissue culture. Cytokinins, particularly 6-benzylaminopurine (BAP), are essential for shoot proliferation and breaking apical dominance (Ab Rahman *et al*., 2015 [1] Arumugam *et al*., 2020 Rosa *et al*., 2015) [4, 35]. BAP is typically used at concentrations ranging from 0.1 to 5.0 mg/L, with optimal shoot multiplication often observed at 0.4-2.0 mg/L (Ab Rahman *et al*., 2015) [1]. Other cytokinins such as kinetin (Kin) and thidiazuron (TDZ) have also been tested, though BAP generally yields superior results for *C. amboinicus* (Dube *et al*., 2011; Rajasekharan, 2010) [11, 32].

Auxins including naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) are employed for callus induction and root development (Simanjuntak *et al*., 2017; Dube *et al*., 2011) [11, 41]. For callus induction, auxins are used either alone or in combination with cytokinins at concentrations of 0.5-3.0 mg/L (Sonappanavar & Jayaraj, 2011; Dube *et al*., 2011) [11, 42]. Root induction is typically achieved using auxins at lower concentrations (0.1-2.0 mg/L) or on auxin-free medium (Ab Rahman *et al*., 2015 ; Simanjuntak *et al*., 2017) [1, 41]. The ratio of auxin to cytokinin is critical in determining the morphogenic response, with high cytokinin to auxin ratios favoring shoot formation and high auxin to cytokinin ratios promoting root or callus formation (Pant, 2014; Saini *et al*., 2011) [36].

4. Callus Induction

Callus induction in *C. amboinicus* can be achieved from various explant types including leaf segments, stem segments, and nodal explants cultured on MS medium supplemented with appropriate combinations of auxins and cytokinins (Roja *et al*., 2006 Sonappanavar & Jayaraj, 2011). [34, 42] Leaf explants are typically cut into 0.5-1.0 cm squared pieces and placed with the abaxial surface in contact with the medium (Sonappanavar & Jayaraj, 2011) [42]. Callus initiation is generally observed within 7-14 days of culture, with callus proliferation continuing over 4-6 weeks (Roja *et al*., 2006 [34] Sonappanavar & Jayaraj, 2011) [42].

The type and concentration of growth regulators significantly influence callus induction frequency and callus characteristics. Combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) or NAA with BAP or kinetin at concentrations

of 1.0-3.0 mg/L are commonly employed (Sonappanavar & Jayaraj, 2011; Dube *et al*., 2011) [11, 42]. Higher auxin concentrations typically promote callus formation, while the addition of cytokinin influences callus texture and color (Pant, 2014; Saini *et al*., 2011) [36]. Callus cultures of *C. amboinicus* have been reported to accumulate essential oils, with some studies showing enhanced thymol content in root-derived callus compared to intact plants (Roja *et al*., 2006) [34].

5. Shoot Proliferation

Shoot proliferation is the most critical stage in micropropagation, determining the multiplication rate and overall efficiency of the protocol. For *C. amboinicus*, optimal shoot multiplication is achieved on MS medium supplemented with BAP at concentrations of 0.4-2.0 mg/L (Ab Rahman *et al*., 2015 [1] Arumugam *et al*., 2020) [4]. Studies have demonstrated that 0.4 mg/L BAP produces the highest frequency of shoot multiplication with well-developed, morphologically normal shoots (Ab Rahman *et al*., 2015) [1]. Higher concentrations of BAP (>2.0 mg/L) or combinations with gibberellic acid (GA3) may result in abnormal plantlet development, including hyperhydricity and fasciation (Ab Rahman *et al*., 2015 Dube *et al*., 2011) [1, 11].

The number of shoots per explant typically ranges from 3 to 15, depending on the concentration of growth regulators and the genotype (Ab Rahman *et al*., 2015 Rajasekharan, 2010) [1, 32]. Shoot multiplication occurs through activation of axillary buds and de novo shoot formation from the explant surface. Subcultures at 4-6week intervals on fresh medium of the same composition maintain high multiplication rates across successive cycles (Arumugam *et al*., 2020 Laslo *et al*., 2011) [4, 25].

6. Root Induction

Root induction is essential for producing self-sustaining plantlets capable of surviving ex vitro conditions. For *C. amboinicus*, rooting is most efficiently achieved on half-strength MS medium (1/2 MS) with or without supplemental auxin (Ab Rahman *et al*., 2015 Arumugam *et al*., 2020) [1, 4]. Indole-3-butyric acid (IBA) at concentrations of 0.5-1.0 mg/L is commonly used for root induction, though spontaneous rooting on hormone-free half-strength MS medium has also been reported (Simanjuntak *et al*., 2017) [41]. Rooting frequency typically ranges from 60% to 100% depending on the auxin concentration and shoot developmental stage (Ab Rahman *et al*., 2015 Arumugam *et al*., 2020) [1, 4].

Roots generally emerge from the base of the shoot within 7-14 days of culture on rooting medium, with well-developed root systems forming after 4 weeks (Simanjuntak *et al*., 2017) [41]. The number of roots per shoot and root length are important parameters for predicting successful acclimatization (Ab Rahman *et al*., 2015) [1]. Well-rooted plantlets with 3-5 roots of 2-3 cm length are optimal for transfer to ex vitro conditions (Arumugam *et al*., 2020) [4].

7. Acclimatization

Acclimatization is the final and often the most challenging stage of micropropagation, involving the transfer of tissue culture plantlets from *in vitro* conditions to the external environment. Rooted plantlets are carefully removed from culture vessels and the agar medium is gently washed from roots using lukewarm water (Arumugam *et al*., 2020; Ab Rahman *et al*., 2015) [1, 4]. Plantlets are then transferred to small pots containing a sterile substrate such as a mixture of peat moss, perlite, and vermiculite (1:1:1 v/v/v) or cocopeat (Ab Rahman *et al*., 2015) [1].

Newly transplanted plantlets require high humidity (>80%) and reduced light intensity during the initial acclimatization period to prevent desiccation stress (Arumugam *et al*., 2020) [4]. This is typically achieved by covering plants with polyethylene bags or maintaining them in humidity chambers for 2-4 weeks, with gradual reduction of humidity over time (Ab Rahman *et al*., 2015 [1] Afolayan & Adebola, 2004) [3]. Temperature is maintained at 25 +/- 2 degrees C throughout acclimatization. Acclimatization success rates of 80-90% have been reported for optimized protocols, with plantlets reaching a stage comparable to conventionally propagated plants within 8-12 weeks (Arumugam *et al*., 2020) [4].

8. Phytochemical Screening Methods

Phytochemical screening of C. amboinicus is conducted using standard qualitative and quantitative methods on extracts prepared from various plant parts (Paul *et al*., 2014; Nagalakshmi *et al*., 2012) [27, 30]. Plant material is typically shade-dried at 40-50 degrees C to constant weight, ground to powder, and extracted using solvents of increasing polarity (petroleum ether, chloroform, ethyl acetate, methanol, and water) through maceration or Soxhlet extraction (Bhattacharjee *et al*., 2010 Paul *et al*., 2014) [9, 30]. Qualitative phytochemical tests employ standard colorimetric and precipitate reactions to detect the presence of major phytochemical classes. Tests for alkaloids use Mayer's, Dragendorff's, and Wagner's reagents; flavonoids are detected using the Shinoda test (magnesium-HCl reduction); tannins are identified with ferric chloride and lead acetate tests; saponins are detected by the foam test and Libermann-Burchard test; terpenoids are identified using the Salkowski test; phenols are detected with ferric chloride solution; and steroids are identified using the Libermann-Burchard test (Paul *et al*., 2014; Nagalakshmi *et al*., 2012) [27, 30].

Quantitative analyses include determination of total phenolic content using the Folin-Ciocalteu reagent with gallic acid as standard, total flavonoid content using the aluminum chloride colorimetric method with quercetin as standard, and total tannin content using the precipitation method (Gupta *et al*., 2013; Bhatt *et al*., 2013) [8, 13]. Essential oil analysis is performed by hydrodistillation using a Clevenger apparatus, with chemical composition determined by gas chromatography-mass spectrometry (GC-MS) (Velasco *et al*., 2009; Arumugam *et al*., 2020) [4, 49].

Results and Discussion

The results presented in this section synthesize findings from studies on *in vitro* propagation of *C. amboinicus* and its phytochemical characterization, integrating both quantitative and qualitative data from published literature.

1. *In vitro* Propagation

The establishment of aseptic cultures is the foundational step in micropropagation, and the success rate varies significantly with the sterilization protocol applied. Studies on *C. amboinicus* consistently demonstrate that nodal segments respond more favorably than leaf or stem explants in terms of both contamination rate and regeneration frequency (Ab Rahman *et al*., 2015 Arumugam *et al*., 2020)

[1, 4]. Treatment with 0.1% HgCl2 for 5-7 minutes has been reported to provide optimal sterilization with minimal phytotoxic effects, achieving contamination rates below 15% and explant survival above 80% (Ab Rahman *et al*., 2015) [1].

Shoot multiplication studies demonstrate that BAP is the most effective cytokinin for *C. amboinicus* micropropagation. At the optimal concentration of 0.4 mg/L BAP, up to 8.3 shoots per explant were produced within 4 weeks of culture, with an average shoot length of 3.5 cm (Ab Rahman *et al*., 2015). Arumugam *et al*. (2020) [1, 4] reported comparable results with a maximum of 12.4 shoots per nodal explant using MS medium supplemented with 1.0 mg/L BAP combined with 0.1 mg/L NAA. The combination of BAP with low concentrations of auxin (NAA or IAA) generally enhances shoot multiplication rates compared to BAP alone, likely due to the synergistic effect of cytokinins and auxins on cell division and organ differentiation (Saini *et al*., 2011; Cho *et al*., 2019) [10, 36].

Root induction from *in vitro* proliferated shoots showed that half-strength MS medium supplemented with 0.5 mg/L IBA was optimal, producing rooting frequencies of 92-100% with an average of 4.2 roots per shoot (Ab Rahman *et al*., 2015 [1] Simanjuntak *et al*., 2017) [41]. Spontaneous rooting was also observed on auxin-free half-strength MS medium in several studies, suggesting that *C. amboinicus* has an inherent tendency for adventitious root formation (Arumugam *et al*., 2020) [4]. The ease of rooting in *C. amboinicus* is consistent with its ready rooting from stem cuttings under conventional propagation (Belniaki *et al*., 2018) [7].

Acclimatization of rooted plantlets to ex vitro conditions proceeded successfully with survival rates of 82-90% when plants were gradually weaned from high humidity conditions over 4-6 weeks (Ab Rahman *et al*., 2015 [1] Arumugam *et al*., 2020) [4]. Plantlets acclimatized on a cocopeat perlite substrate (2:1) showed superior survival and growth compared to those on garden soil or vermiculite-based substrates. After 8 weeks in the greenhouse, acclimatized plants were morphologically indistinguishable from conventionally propagated plants, confirming the normality of tissue culture-derived plants (Arumugam *et al*., 2020) [4].

Notably, analysis of essential oil content in tissue culture-derived plants confirmed that micropropagated *C. amboinicus* plants maintained essential oil profiles comparable to field-grown specimens. GC-MS analysis of essential oils from micropropagated plants revealed carvacrol (38-55%) and thymol (15-30%) as the major components, consistent with the composition reported for wild-type plants (Arumugam *et al*., 2020 Roja *et al*., 2006) [4, 34]. Callus-derived tissues showed variable essential oil compositions, with root-derived callus showing particularly enhanced thymol accumulation (Roja *et al*., 2006), suggesting tissue-specific differences in essential oil biosynthesis.

2. Phytochemical Constituents

Qualitative phytochemical screening of *C. amboinicus* consistently reveals the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, steroids, and glycosides across multiple studies using different solvent extracts (Paul *et al*., 2014; Nagalakshmi *et al*., 2012; Laungsuwon, 1997) [26, 27, 30]. The presence of these major phytochemical classes has been confirmed in both field-grown and tissue culture-derived plants, validating the use of micropropagated material for phytochemical production (Arumugam *et al*., 2020 Varnika *et al*., 2020) [4, 47].

Quantitative analysis of phenolic compounds and flavonoids reveals significant variation based on solvent polarity, plant part, and collection conditions. Total phenolic content ranges from 15.2 to 89.4 mg GAE/g dry weight across different extract types, with methanol extracts consistently showing the highest phenolic content (Gupta *et al*., 2013; Bhatt *et al*., 2013) [8, 13]. Total flavonoid content ranges from 8.3 to 45.6 mg QE/g dry weight, with ethanolic extracts showing maximum flavonoid content (Gurning, 2020 Gurning, 2020) [14, 15]. The correlation between total phenolic content and antioxidant activity is consistently positive across studies, confirming the contribution of phenolics to the antioxidant properties of the plant (Suryowati *et al*., 2015a; Suryowati *et al*., 2015b) [43, 44].

GC-MS analysis of the essential oil of *C. amboinicus* identifies carvacrol and thymol as the predominant components, with relative contents varying by geographic origin, season, and plant part analyzed (Velasco *et al*., 2009; Suryowati *et al*., 2015a) [43, 44, 49]. Carvacrol typically constitutes 40-60% of the total essential oil, while thymol content ranges from 10-30% (Velasco *et al*., 2009; Arumugam *et al*., 2020) [4, 49]. Other significant components include p-cymene, gamma-terpinene, sabinene, beta-caryophyllene, and eugenol (Velasco *et al*., 2009; Bhatt *et al*., 2013) [8, 49].

Specific phytochemical compounds identified through chromatographic and spectroscopic analyses include rosmarinic acid, caffeic acid, luteolin, apigenin, quercetin, kaempferol, and various phenolic acids (Gupta *et al*., 2013; Bhatt *et al*., 2013 [8, 13] Suttisri & Laungsuwon, 1998). Laila *et al*. (2020) [24] identified unique phytochemical constituents from pressurized liquid extraction fractions, including novel terpenoids and glycosides not previously reported for *C. amboinicus*. Monoterpene glycosides and furan derivatives have been isolated and characterized by Suttisri and Laungsuwon (1998), adding to the chemical diversity of this species. Phytochemical and molecular analyses of Coleus cultivars also revealed significant variation in secondary metabolite profiles across genotypes (Shoaib *et al*., 2020) [39].

Biological and Pharmacological Activities

1. Antimicrobial Activity

Antimicrobial activity is one of the most extensively studied biological properties of *C. amboinicus*, with demonstrated efficacy against a broad range of bacterial and fungal pathogens. Studies using disc diffusion, broth microdilution, and agar dilution methods consistently report inhibitory activity against gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes) and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae) (Paul *et al*., 2014; Nagalakshmi *et al*., 2012; Kumar *et al*., 2008) [23, 27, 30].

Minimum inhibitory concentrations (MICs) for methanol and ethanol extracts against common pathogens range from 0.25 to 4.0 mg/mL, with gram-positive bacteria generally showing greater susceptibility than gram-negative bacteria (Gurgel *et al*., 2009 [17] Khattak *et al*., 2013) [21]. Essential oil of *C. amboinicus* exhibits potent antimicrobial activity with MICs of 0.15-0.78 mg/mL against S. aureus, including

methicillin-resistant strains (MRSA) (Vasconcelos *et al*., 2017; Gurgel *et al*., 2009) [17, 48]. The antimicrobial activity is primarily attributed to carvacrol and thymol, which disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with cellular metabolic processes (Vasconcelos *et al*., 2017 Velasco *et al*., 2009) [48, 49]. Bioassay-guided isolation studies have identified specific antimicrobial compounds in *C. amboinicus*, including sesquiterpenes and phenolic compounds (Kraus *et al*., 2004 [22] Khattak & Taher, 2011) [20]. Antimicrobial activity has also been reported from endophytic fungi isolated from C. amboinicus leaves (Astuti *et al*., 2014; Astuti *et al*., 2020) [5, 6].

2. Antioxidant Activity

C. amboinicus exhibits potent antioxidant activity, demonstrated through multiple *in vitro* assays including DPPH radical scavenging, FRAP, ABTS, and lipid peroxidation inhibition (Gurning, 2020a; Gurning, 2020b; Gupta *et al*., 2013; Bhattacharjee *et al*., 2010). Gurning 2020 [9, 13, 14, 15] demonstrated that methanol extracts of C. amboinicus leaves exhibited strong DPPH radical scavenging activity with IC50 values ranging from 50 to 150 ug/mL, comparable to standard antioxidants such as ascorbic acid. The ethyl acetate fraction showed particularly high antioxidant activity, attributed to enrichment in phenolic compounds (Gurning, 2020) [14, 15]. A cytoprotective study by Haryani *et al*. (2018) [18] confirmed that ethanol fraction of *C. amboinicus* protected Vero cells from H2O2-induced oxidative damage, further validating its antioxidant potential at the cellular level.

3. Anti-inflammatory and Other Activities

The anti-inflammatory properties of C. amboinicus have been documented through protein denaturation inhibition, erythrocyte membrane stabilization, and enzyme inhibition assays (Bhattacharjee *et al*., 2010 [9] Gurgel, 2007) [16]. The essential oil components, particularly carvacrol and thymol, contribute to the anti-inflammatory activity through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (Gurgel, 2007) [16]. Anti-hyperuricemic activity has been demonstrated by Suryowati and Gultom (2018) [45], showing significant reduction of serum uric acid levels in subjects with hyperuricemia following *C. amboinicus* extract supplementation.

Cytotoxic activity against HeLa and Vero cell lines has been reported by Hasibuan (2017) [19], with n-hexane and ethanol extracts showing selective toxicity toward cancer cells. The selective cytotoxicity suggests potential for development of anticancer agents (Hasibuan, 2017) [19]. Additional pharmacological activities include anthelmintic effects (Bhattacharjee *et al*., 2010) [9], antimicrobial activity against Candida albicans (Rianti & Agustantina, 2008) [33], modulation of rumen fermentation and methanogenesis (Yanza *et al*., 2018; Adriani *et al*., 2019) [2], and nutraceutical potential from stem extracts (Bhatt *et al*., 2013) [8]. The comprehensive pharmacological profile of *C. amboinicus* validates its traditional medicinal uses and supports its development as a pharmaceutical and nutraceutical resource (Silva *et al*., 2020; Petersen, 1994) [31, 40].

Conclusion

This comprehensive review has synthesized current knowledge on the *in vitro* propagation and phytochemical characterization of *Coleus amboinicus*, a valuable medicinal plant with diverse therapeutic applications. The establishment of efficient micropropagation protocols represents a significant advancement in addressing conservation challenges and commercial production needs for this species. The optimal protocol involves culture of nodal explants on MS medium supplemented with 0.4 mg/L BAP for shoot multiplication, followed by rooting on half-strength MS medium with or without auxin supplementation, and gradual acclimatization over 4-8 weeks. These protocols achieve multiplication rates of 8-12 shoots per explant with acclimatization success rates exceeding 80%, demonstrating the technical and economic feasibility of large-scale micropropagation.

The maintenance of essential oil profiles and phytochemical composition in tissue culture-derived plants is a critical finding validating the use of micropropagation for commercial production of *C. amboinicus*. The comparable or even enhanced accumulation of certain compounds such as thymol in tissue cultures suggests potential for using *in vitro* systems not only for plant propagation but also for targeted production of valuable phytochemicals. This dual application enhances its value for sustainable utilization.

The rich phytochemical profile of *C. amboinicus*, encompassing alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, and essential oils dominated by carvacrol and thymol, provides a strong scientific foundation for its traditional medicinal uses. The demonstrated antimicrobial activity against a broad spectrum of pathogens, including antibiotic-resistant strains and biofilm-forming bacteria, positions *C. amboinicus* as a promising source of novel antimicrobial agents. The potent antioxidant and anti-inflammatory activities, along with cytotoxic effects against cancer cells, further expand the pharmaceutical potential of this plant.

The integration of biotechnological approaches with phytochemical research offers promising avenues for the sustainable development of *C. amboinicus* as a medicinal plant resource. Future research should focus on molecular characterization of biosynthetic pathways for key bioactive compounds, optimization of tissue culture conditions for enhanced secondary metabolite production, genetic transformation for trait improvement, and comprehensive clinical studies to validate traditional uses. The protocols and knowledge synthesized in this review provide a roadmap for researchers, cultivators, and pharmaceutical developers interested in harnessing the therapeutic potential of this remarkable medicinal plant while ensuring its conservation for future generations.

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