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In vitro PROPAGATION AND PHYTOCHEMICAL STUDIES IN *Hemigraphis colorata* (Blume) H.G. HALLIER: A REVIEW

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ABSTRACT

Hemigraphis colorata (Blume) H.G. Hallier, synonymously reported in parts of the literature as Hemigraphis alternata (Burm.f.) T. Anderson, is a low-growing perennial herb of the family Acanthaceae. It is widely cultivated as an ornamental plant and is traditionally used for wound healing, inflammation, skin disorders, piles, dysentery and other ailments. Increasing interest in this species has resulted from its reported antimicrobial, antioxidant, anti-inflammatory, antidiabetic and wound-healing properties. This review summarizes available information on the in vitro propagation and phytochemical profile of H. colorata. Nodal explants cultured on Murashige and Skoog medium supplemented with cytokinins, particularly 6-benzylaminopurine, provide an effective route for rapid clonal multiplication. A combination of benzylaminopurine and a-naphthalene acetic acid enhances multiple-shoot production, whereas half-strength medium containing indole-3-butyric acid promotes rooting. Preliminary phytochemical investigations indicate the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, sterols, carbohydrates, glycosides and related compounds. However, much of the phytochemical evidence remains based on qualitative screening, and further chromatographic, spectroscopic and pharmacological validation is required. Integrating micropropagation with metabolite profiling may support the conservation, standardization and future pharmaceutical development of this medicinally important species.

KEYWORDS: *Hemigraphis Colorata, Hemigraphis Alternata, Micropropagation, Nodal Explant, Phytochemical Screening, Flavonoids, Phenolics, Medicinal Plants, Wound Healing, Metabolomics.*

INTRODUCTION

Medicinal plants remain important sources of traditional remedies and new bioactive molecules. Among underutilized medicinal herbs, *Hemigraphis colorata* has attracted attention because of its ethnomedicinal applications and ease of vegetative growth. The plant is commonly known as murikooti or murian pacha in parts of Kerala and as metal leaf, red flame ivy, waffle plant or Java ivy in horticultural contexts. It is a creeping herb with attractive metallic-purple foliage and rooting stems, making it valuable both as an ornamental and medicinal plant (Minol 2023; Anjala Jabeen et al. 2026; Rex Devasahayam Arokia Balaya et al. 2024).

Traditionally, fresh leaves are crushed and applied to cuts and wounds. Reports also describe the use of the plant for inflammation, ulcers, piles, bloody dysentery, excessive menstruation and certain skin complaints. The plant has been investigated in experimental models for wound healing, antimicrobial, antioxidant, anti-inflammatory, antidiabetic and anthelmintic activities. These effects are generally attributed to its phenolic compounds, flavonoids, tannins, terpenoids and other secondary metabolites, although the contribution of individual compounds has not been fully established (Minol 2023; Rex Devasahayam Arokia Balaya et al. 2024; Treesa Joy et al. 2024; Anjala Jabeen et al. 2026).

The natural propagation of *H. colorata* occurs readily through creeping stems and nodes. Nevertheless, conventional propagation may be inadequate when uniform, disease-free and high-volume planting material is required. In vitro propagation provides a controlled alternative capable of producing genetically uniform plantlets throughout the year. It may also provide a foundation for future studies on elicitation, cellular production of secondary metabolites and conservation of selected chemotypes (Nair and Nair 2018; Anju and Thomas 2019).

Botanical and Medicinal Importance

Hemigraphis colorata is a small, prostrate or creeping perennial herb generally reaching approximately 15–30 cm in height. Its opposite leaves are ovate to cordate, serrated or crenate, and typically display a metallic purple upper surface with a darker purple lower surface. The plant produces small whitish flowers with faint purple markings in terminal spikes (Minol 2023; Rex Devasahayam Arokia Balaya et al. 2024).

Pharmacognostic studies have reported diagnostic leaf characters that may assist in authentication and quality control. These include diacytic stomata, multicellular non-glandular trichomes, a single-layered epidermis, differentiated palisade and spongy tissues, and clearly defined vascular bundles. One study reported an upper epidermal stomatal index of 8.65, a lower epidermal stomatal index of 22.34 and a palisade ratio of 2.75. Such anatomical and microscopic parameters can help distinguish genuine leaf material from adulterants (Priya et al. 2021; Priya 2021).

The traditional medicinal importance of the plant is primarily associated with its leaves. Leaf paste or juice is used externally to control bleeding and promote wound repair. Experimental studies have reported enhanced wound contraction and epithelialization in animal models, while extracts have also shown antimicrobial and antioxidant effects. These findings provide a scientific basis for further

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investigation but should not be interpreted as evidence of established clinical efficacy (Rex Devasahayam Arokia Balaya et al. 2024; Treesa Joy et al. 2024; Minol 2023; Shameela Nasrin and Joseph 2022).

In Vitro Propagation

Rationale for Micropropagation

Micropropagation is particularly useful for medicinal plants that are required in large numbers or whose active constituents may vary according to genotype, environment and developmental stage. It can provide:

- Rapid multiplication of elite or high-yielding plants.
- Production of relatively uniform planting material.
- Year-round propagation independent of seasonal conditions.
- Reduced transfer of soil-borne pathogens when cultures are properly established.
- A controlled system for studying growth regulators and metabolite production.

Because *H. colorata* is usually propagated by stem cuttings, nodal culture is an appropriate approach. Nodal segments contain pre-existing axillary meristems and generally have a lower risk of genetic variation than indirect regeneration through callus (Nair and Nair 2018; Anju and Thomas 2019).

Explant Establishment

Young and healthy shoots are suitable sources of nodal explants. In the reported protocol, leaves were removed and stems were cut into small segments containing at least one node. Explants were washed under running water, treated with detergent, pretreated with fungicide and surface sterilized before inoculation on culture medium. The second and third nodes were found to be particularly suitable for culture initiation (Nair and Nair 2018).

Cultures were established on Murashige and Skoog medium containing 3% sucrose and solidified with agar. The medium pH was adjusted to approximately 5.8, and cultures were maintained under a 12-hour photoperiod at approximately 23 ± 2°C. Shoot-bud emergence occurred within four to five days after inoculation, indicating a rapid response of the nodal meristem (Nair and Nair 2018).

The use of mercuric chloride in older protocols deserves caution because of its toxicity and environmental persistence. Future protocols should evaluate safer sterilants, such as sodium hypochlorite, calcium hypochlorite, hydrogen peroxide or plant-preservative mixtures, while maintaining acceptable contamination control (Nair and Nair 2018; Anju and Thomas 2019).

Shoot Induction and Multiplication

Cytokinin treatment is central to shoot induction. Benzylaminopurine, commonly abbreviated as BA or BAP, was more effective than kinetin in inducing shoots from nodal explants. On MS medium supplemented with 3 µM BAP, the study reported an 87% regeneration response, with an average of 10.39 shoots per explant and a mean shoot length of 4.72 cm after 60 days (Nair and Nair 2018).

Repeated subculture on medium containing BAP and a low concentration of NAA further improved multiplication. The most effective treatment consisted of 3 µM BAP with 0.75 µM NAA, producing approximately 11.61 shoots per explant and an 86% regeneration response after 60 days. The improved response demonstrates the importance of auxin–cytokinin balance rather than cytokinin concentration alone (Nair and Nair 2018).

Rooting and Acclimatization

Root induction was most successful on half-strength MS medium containing indole-3-butyric acid. A concentration of 2 µM IBA produced approximately 98% rooting, with a mean of 19.63 roots per shoot after four weeks. NAA also supported rooting but was generally less effective than IBA under the reported conditions (Nair and Nair 2018).

Rooted plantlets were transferred to sterile vermiculite and maintained under high humidity using transparent covers. Gradual reduction of humidity was necessary before transfer to soil. Approximately 97% of the plantlets survived hardening and subsequent transfer to garden soil. The acclimatized plants showed normal growth and morphology, suggesting that the protocol is suitable for large-scale clonal propagation (Nair and Nair 2018).

Summary of the Reported Protocol

Propagation Stage	Medium or treatment	Main response
Culture Initiation	MS medium with BAP or kinetin	Shoot buds emerged within four to five days
Shoot Induction	MS + 3 µM BAP	87% response; approximately 10.39 shoots per explant
Multiple-Shoot Formation	MS + 3 µM BAP + 0.75 µM NAA	Approximately 11.61 shoots per explant; 86% response
Rooting	Half-strength MS + 2 µM IBA	Approximately 98% rooting; about 19.63 roots per shoot
Acclimatization	Vermiculite followed by soil transfer	Approximately 97% survival

Although the protocol is promising, it should be interpreted carefully because the cited study used the name *Hemigraphis alternata*. Since taxonomic names have been used inconsistently in the literature, future research should verify plant identity using authenticated herbarium vouchers and, where possible, molecular barcoding (Nair and Nair 2018; Rex Devasahayam Arokia Balaya et al. 2024; Treesa Joy et al. 2024).

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Phytochemical Studies

Preliminary Screening

Phytochemical screening of *H. colorata* leaves has detected a broad range of primary and secondary metabolites. Reported groups include:

- Alkaloids.
- Flavonoids and flavones.
- Phenolic compounds.
- Tannins and phlobatannins.
- Saponins.
- Terpenoids.
- Steroids and sterols.
- Coumarins.
- Carbohydrates and reducing sugars.
- Glycosides.
- Proteins and amino-acid-related compounds.
- Resins and quinone-related constituents.

The presence or absence of a compound class varies among studies. Such variation is expected because phytochemical results depend on plant part, developmental stage, geographical origin, drying conditions, extraction solvent, extraction method and the sensitivity of the chemical test (Priya et al. 2021; Minol 2023; Anjala Jabeen et al. 2026; Dalia Purushothaman and Chandra 2021).

A detailed study using acetone, methanol and water extracts detected alkaloids, carbohydrates and phenols in all three extracts. Flavonoids were detected more consistently in methanolic and aqueous extracts, while steroids and terpenoids were more evident in polar extracts under some test systems. Saponins and tannins were not consistently detected in that investigation, despite being reported in other studies. These differences show why preliminary screening should be considered indicative rather than definitive (Priya et al. 2021; Syamili et al. 2020).

Solvent Effects

Extraction solvent strongly influences the apparent phytochemical profile. Polar solvents such as water and methanol are generally more suitable for extracting phenolic compounds, flavonoid glycosides, carbohydrates and certain alkaloids. Less-polar solvents, such as hexane, preferentially extract lipophilic compounds, including hydrocarbons, sterols, pigments and terpenoid constituents (Priya et al. 2021; Safna et al. 2020; Minol 2023).

Reported studies have identified phenolic acids or related phenolic derivatives such as chlorogenate, cinnamate, coumarate, gallate and ferulate in discussions of the plant's antioxidant potential. However, the exact identity and concentration of these compounds require confirmation using validated chromatographic methods. Statements based only on colour reactions or nonspecific screening should not be treated as compound-level identification (Priya et al. 2021; Dalia Purushothaman and Chandra 2021; Minol 2023).

Quantitative Phytochemical Estimates

Quantitative studies have provided estimates of total phenolic, flavonoid and related contents. One investigation reported total phenolic contents of approximately 315 mg gallic acid equivalent per gram in ethanolic extract and 440 mg GAE/g in aqueous extract. Total flavonoid contents were estimated at 13.68 mg rutin equivalent per gram in ethanolic extract and 7.43 mg RE/g in aqueous extract. Total flavonol contents were 6.27 mg RE/g in ethanolic extract and 3.15 mg RE/g in aqueous extract. Total tannin content was reported at 162.18 mg tannic acid equivalent per gram in methanolic extract. Total antioxidant activity was found to be 432.33 mg/g in *H. colorata* methanolic extract (Dalia Purushothaman and Chandra 2021; Shameela Nasrin and Joseph 2022).

These quantitative data support the qualitative screening results and provide a basis for comparing extracts from different solvents, plant parts or growth conditions. However, the values should be interpreted with caution because they depend on the specific assay conditions, standards and calibration methods used (Dalia Purushothaman and Chandra 2021; Shameela Nasrin and Joseph 2022).

Chromatographic Evidence

Compared with preliminary screening, chromatographic studies provide more specific information. GC–MS analysis of a hexane extract identified squalene as a major phytochemical compound. The same study reported antibacterial, antioxidant and antielastase activity for the extract, linking a lipophilic fraction with several biological effects (Safna et al. 2020; Dalia Purushothaman and Chandra 2021).

GC–MS analysis of methanolic extract revealed 17 major peaks, indicating the presence of multiple bioactive compounds. LC–MS/MS-based global metabolomics of aqueous and ethanolic leaf extracts identified 2285 metabolites from 24,203 spectra, classified under ketones, carboxylic acids, primary aliphatic amines, steroids and steroid derivatives, among others. Targeted multiple reaction monitoring confirmed the presence of curcumin in both aqueous and ethanolic extracts at approximately 32 ng per milligram of leaf extract (Dalia Purushothaman and Chandra 2021; Rex Devasahayam Arokia Balaya et al. 2024).

This finding is important because it demonstrates that *H. colorata* contains not only polar phenolics and flavonoids but also potentially important nonpolar metabolites. Nevertheless, the detection of squalene or another compound by GC–MS does not by itself prove that the compound is responsible for a biological effect. Bioassay-guided fractionation, authentic standards, quantitative analysis and mechanistic studies are needed to establish causation (Safna et al. 2020; Rex Devasahayam Arokia Balaya et al. 2024; Dalia Purushothaman and Chandra 2021).

Relationship to Biological Activity

The reported phytochemical groups provide plausible explanations for the biological activities observed in experimental studies:

- Phenolics and flavonoids may contribute to free-radical-scavenging and antimicrobial activity.
- Tannins may contribute to astringency, protein precipitation and local wound effects.
- Terpenoids and sterols may participate in anti-inflammatory and membrane-associated activities.
- Alkaloids and glycosides may contribute to antimicrobial or metabolic effects, although their identities remain insufficiently characterized.

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- Saponins may influence membrane permeability and exhibit antimicrobial or anti-inflammatory properties.

The relationship between phytochemistry and pharmacology remains largely correlative. For example, a positive ferric-chloride test indicates phenolic substances but does not identify a specific phenol or establish its pharmacological potency. Similarly, antioxidant assays such as DPPH measure chemical radical-scavenging capacity in vitro and cannot be directly equated with antioxidant effects in humans (Priya et al. 2021; Dalia Purushothaman and Chandra 2021; Minol 2023; Rex Devasahayam Arokia Balaya et al. 2024).

Methodological Considerations

Future phytochemical work should move beyond preliminary colour tests. A stronger research design would include authenticated plant material, clear documentation of collection site and season, standardized drying and extraction, determination of extraction yield, and quantitative estimation of total phenolic and flavonoid contents (Dalia Purushothaman and Chandra 2021; Shameela Nasrin and Joseph 2022; Rex Devasahayam Arokia Balaya et al. 2024).

High-performance liquid chromatography, liquid chromatography–mass spectrometry, nuclear magnetic resonance spectroscopy and validated GC–MS methods should be used to characterize individual metabolites. Chemical fingerprints should be compared across leaves, stems, roots, in vitro shoots and acclimatized plants. This would help determine whether micropropagated plants retain the chemical profile of donor plants (Rex Devasahayam Arokia Balaya et al. 2024; Dalia Purushothaman and Chandra 2021).

Quality control should also include genetic fidelity testing. Techniques such as inter-simple sequence repeat, random amplified polymorphic DNA or simple sequence repeat analysis can help detect somaclonal variation. Where medicinal use is intended, microbial limits, heavy metals, pesticide residues, residual solvents and acute and subacute toxicity should be evaluated (Nair and Nair 2018; Anju and Thomas 2019).

The relationship between culture conditions and secondary metabolism deserves special attention. Light intensity, sucrose concentration, nitrogen form, elicitors, precursor feeding and plant growth regulators may influence phenolic and terpenoid accumulation. In vitro cultures could therefore serve not only for plant multiplication but also as experimental systems for improving the production of selected metabolites (Nair and Nair 2018; Rex Devasahayam Arokia Balaya et al. 2024).

Research Gaps and Future Prospects

Several gaps are evident in the current literature:

- Taxonomic inconsistency between *H. colorata* and *H. alternata* complicates comparison among studies (Rex Devasahayam Arokia Balaya et al. 2024; Treesa Joy et al. 2024; Minol 2023).
- Many reports use qualitative phytochemical tests without quantitative confirmation (Priya et al. 2021; Syamili et al. 2020; Dalia Purushothaman and Chandra 2021).
- Few studies compare the metabolite profiles of field-grown and micropropagated plants (Nair and Nair 2018; Anju and Thomas 2019).
- The active principles responsible for wound healing and antimicrobial effects remain incompletely defined (Rex Devasahayam Arokia Balaya et al. 2024; Treesa Joy et al. 2024).
- Standardized extraction methods and validated chemical markers have not been universally established (Dalia Purushothaman and Chandra 2021; Shameela Nasrin and Joseph 2022).
- Evidence is dominated by in vitro assays and small animal studies, with limited clinical validation (Rex Devasahayam Arokia Balaya et al. 2024; Treesa Joy et al. 2024; Minol 2023).
- The safety, dosage, pharmacokinetics and long-term toxicity of internal preparations require further investigation (Minol 2023; Anjala Jabeen et al. 2026).

An integrated research program should therefore combine authenticated germplasm, optimized micropropagation, genetic-fidelity assessment, metabolomic profiling and bioassay-guided isolation. A particularly useful approach would be to compare donor plants, in vitro shoots, rooted plantlets and field-acclimatized plants for total phenolics, flavonoids, squalene and other candidate markers. Such work could establish whether micropropagation is suitable for the production of chemically consistent medicinal raw material (Nair and Nair 2018; Rex Devasahayam Arokia Balaya et al. 2024; Dalia Purushothaman and Chandra 2021; Anju and Thomas 2019).

Conclusion

Hemigraphis colorata is a promising medicinal and ornamental herb with considerable potential for standardized cultivation and pharmaceutical research. Nodal-explant culture on MS medium, particularly with BAP for shoot induction, BAP plus low NAA for shoot multiplication and IBA for rooting, provides an efficient method for rapid clonal propagation. The reported protocol achieved approximately 11.61 shoots per explant, 98% rooting and 97% acclimatization survival under optimized conditions (Nair and Nair 2018).

Phytochemical studies indicate the presence of diverse metabolite classes, including phenolics, flavonoids, alkaloids, tannins, saponins, terpenoids, steroids and sterols. Chromatographic evidence has additionally identified squalene as a major constituent of a hexane extract and curcumin in aqueous and ethanolic extracts (Safna et al. 2020; Rex Devasahayam Arokia Balaya et al. 2024; Dalia Purushothaman and Chandra 2021). However, the current evidence base requires improved taxonomic authentication, quantitative chemical analysis, genetic-fidelity testing and pharmacological validation. The combination of micropropagation and modern phytochemical profiling could support conservation, quality control and future development of *H. colorata*-based herbal products (Nair and Nair 2018; Rex Devasahayam Arokia Balaya et al. 2024; Dalia Purushothaman and Chandra 2021; Anju and Thomas 2019).

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