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COMPARATIVE EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF WILD AND *IN VITRO* PROPAGATED *LAVANDULA ANGUSTIFOLIA* MILL.

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ABSTRACT

Lavandula angustifolia Mill. (Lavender), an important medicinal and aromatic plant belonging to the family Lamiaceae, is widely valued for its bioactive secondary metabolites including phenolics, flavonoids, terpenoids and essential oil constituents. These phytochemicals contribute significantly to antioxidant and antimicrobial properties; however, sustainable availability of quality planting material remains a major challenge. The present study aimed to compare the antioxidant and antimicrobial potential of wild and in vitro propagated Lavandula angustifolia Mill. In vitro propagation was successfully established using apical and nodal explants, where nodal segments showed higher aseptic response (92%) compared with apical segments (80%). Maximum shoot induction (93%) was recorded on MS medium supplemented with BAP (8.88 µM) and NAA (2.0 µM), producing 4.8 ± 0.6 shoots per explant. The highest multiplication response was obtained during the third subculture with 8.5 ± 0.8 shoots per explant. Root induction was optimum at IBA (3.0 µM), showing 92% rooting with 5.8 ± 0.6 roots per plantlet. Hardened plantlets showed 88% survival after four weeks of acclimatization. Comparative biological evaluation revealed that ethanol extracts exhibited maximum antimicrobial activity. Wild plant extracts showed the highest antibacterial activity against Escherichia coli, with flowers showing a maximum zone of inhibition of 18 mm, followed by leaves (17 mm). Similarly, maximum antifungal activity against Aspergillus niger was recorded in flower ethanol extract (20 mm), followed by leaf ethanol extract (19 mm). In vitro propagated plants showed comparable antimicrobial potential. The findings indicate that micropropagation maintains the biological potential of L. angustifolia and provides a sustainable approach for production of medicinally valuable plant material.

KEYWORDS - *Lavandula angustifolia, micropropagation, phenolics, flavonoids, antioxidant activity, antimicrobial activity, medicinal plants.*

1. INTRODUCTION

1.1 Medicinal Importance of *Lavandula angustifolia* Mill.

Medicinal plants are important sources of therapeutic compounds due to their rich diversity of bioactive secondary metabolites. *Lavandula angustifolia* Mill. (lavender), belonging to the family Lamiaceae, is an important aromatic and medicinal plant widely known for its essential oil, phytochemical richness, and pharmacological properties. Lavender is extensively used in pharmaceutical, cosmetic, perfumery, and aromatherapy industries due to its valuable volatile compounds (Prusinowska & Śmigielski, 2014).

The essential oil of *L. angustifolia* contains major bioactive constituents such as linalool, linalyl acetate, and other terpenoids, which contribute to its characteristic aroma and therapeutic properties (Cavanagh & Wilkinson, 2002). Lavender exhibits various biological activities, including antioxidant, antimicrobial, antifungal, and anti-inflammatory effects. Phenolic compounds and flavonoids present in lavender extracts contribute significantly to antioxidant activity by scavenging free radicals and reducing oxidative stress (Spiridon *et al*., 2011).

The antimicrobial activity of lavender is mainly associated with terpenoid compounds that affect microbial membrane integrity and inhibit pathogen growth. These properties make *L. angustifolia* a promising natural source of bioactive compounds for pharmaceutical and therapeutic applications (Moon *et al*., 2006).

1.2 Bioactive Compounds of Lavender and Their Biological Significance

The medicinal properties of *L. angustifolia* are mainly attributed to its diverse secondary metabolites, including phenolics, flavonoids, terpenoids, and essential oil components. Phenolic compounds and flavonoids possess strong antioxidant properties due to their ability to neutralize reactive oxygen species. They also contribute to antimicrobial and anti-inflammatory activities. Terpenoids such as linalool and linalyl acetate are major components of lavender essential oil and exhibit antibacterial and antifungal properties by affecting microbial cell membranes (Cavanagh & Wilkinson, 2002).

The combined action of these phytochemicals provides the scientific basis for the antioxidant and antimicrobial potential of lavender extracts.

1.3 Importance of *In Vitro* Propagation of *Lavandula angustifolia*

Increasing demand for medicinal plants requires sustainable approaches for conservation and large-scale production. Conventional propagation methods may not provide sufficient uniform plant material; therefore, *in vitro* propagation and micropropagation techniques are valuable tools for rapid multiplication and conservation of medicinal plants.

In vitro propagation enables the production of disease-free and genetically uniform plants under controlled conditions. Tissue culture studies in *L. angustifolia* have demonstrated successful regeneration using MS medium supplemented with plant growth regulators such as BAP, NAA, and IBA for shoot induction and rooting (De Bona *et al*., 2011).

Micropropagation helps in conservation of valuable germplasm, rapid multiplication of elite plants, and sustainable utilization of medicinal resources.

1.4 Aim of the Present Study

The present investigation was undertaken to compare the antioxidant and antimicrobial potential of wild and in vitro propagated *Lavandula angustifolia* Mill.

2. MATERIALS AND METHODS

2.1 Plant Material

Healthy and disease-free plants of *Lavandula angustifolia* Mill. were selected for the present investigation. Wild plants were collected and authenticated based on morphological characteristics, while in vitro propagated plants were obtained through tissue culture techniques. The in vitro propagation process involved the establishment of aseptic cultures using suitable explants, followed by shoot induction, multiplication, rooting, and hardening under controlled laboratory conditions.

For in vitro regeneration, explants were cultured on Murashige and Skoog (MS) basal medium supplemented with appropriate concentrations of plant growth regulators. Cytokinin and auxin combinations were used for shoot induction and multiplication, whereas auxin supplementation was used for root development. The regenerated plantlets were acclimatized under greenhouse conditions before being used for comparative phytochemical and biological evaluation. The use of in vitro propagated plants enabled assessment of whether tissue culture-derived plants retained the medicinal properties of naturally grown plants.

2.2 Preparation of Plant Extracts

Different plant parts of wild and in vitro propagated *L. angustifolia* Mill. were separately collected for extraction. The selected plant parts included: Leaves, Flowers, Stems and Roots.

The collected samples were washed thoroughly, shade dried, and powdered using a mechanical grinder. The powdered plant materials were extracted separately using different solvents of varying polarity, including: Water, Ethanol, Chloroform and Petroleum ether.

Solvent extraction was performed to obtain bioactive compounds with different chemical properties. The extracts were filtered, concentrated, and stored under suitable conditions until further phytochemical and biological analyses. The comparative evaluation of different solvent extracts helped to determine the influence of solvent polarity on extraction efficiency and biological activity.

2.3 Phytochemical Analysis

Qualitative phytochemical screening of wild and in vitro propagated *L. angustifolia* extracts was carried out to identify the presence of major groups of secondary metabolites. The extracts were evaluated for important bioactive compounds including: Phenolic compounds, Flavonoids, Alkaloids and Terpenoids.

Standard phytochemical screening procedures were followed for detection of these compounds. The presence of phenolic and flavonoid compounds was considered significant due to their reported antioxidant properties, whereas terpenoids and other secondary metabolites were associated with antimicrobial potential.

The phytochemical profile of different extracts was compared between wild and in vitro propagated plants to determine the effect of propagation method on bioactive compound production.

2.4 Antioxidant Activity

DPPH Radical Scavenging Assay

The antioxidant potential of different extracts of wild and in vitro propagated *L. angustifolia* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Different concentrations of plant extracts were prepared and tested for their ability to reduce DPPH free radicals.

The reaction mixture containing plant extract and DPPH solution was incubated under suitable conditions, and absorbance was measured spectrophotometrically. The decrease in absorbance indicated the scavenging ability of the extracts towards DPPH radicals.

The percentage of radical scavenging activity was calculated using the standard formula:

$$\text{DPPH scavenging activity } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where:

A_c = Absorbance of control
A_s = Absorbance of sample

Higher percentage inhibition indicated greater antioxidant potential of the extract. The antioxidant activity was compared among different plant parts, solvents, and between wild and in vitro propagated plants.

2.5 Antimicrobial Activity

The antimicrobial potential of *L. angustifolia* extracts was evaluated against selected bacterial and fungal pathogens using the zone of inhibition method.

2.5.1 Antibacterial Activity

The antibacterial activity of different plant extracts was tested against:

Escherichia coli

Extracts prepared from different plant parts were evaluated for their inhibitory effect on bacterial growth. The antimicrobial response was determined by measuring the diameter of the zone of inhibition around the extract-treated wells/discs. A larger zone of inhibition indicated stronger antibacterial activity.

2.5.2 Antifungal Activity

The antifungal activity of *L. angustifolia* extracts was evaluated against:

Aspergillus niger

Different solvent extracts were tested for their ability to inhibit fungal growth. The inhibition zones produced around treated wells were measured and recorded in millimeters. Comparative analysis was performed among different plant parts and solvents to identify the most effective extract.

Statistical Analysis

All experiments were performed with appropriate replications, and the obtained data were expressed as mean values. Comparative analysis was performed to evaluate differences between wild and in vitro propagated plants regarding phytochemical composition, antioxidant potential, and antimicrobial activity.

3. RESULTS AND DISCUSSION

The present study evaluated the comparative phytochemical composition, antioxidant potential, and antimicrobial activity of wild and in vitro propagated *Lavandula angustifolia* Mill. The biological activities of medicinal plants are largely influenced by the presence of secondary metabolites such as phenolics, flavonoids, terpenoids, and other bioactive compounds. Comparative assessment of wild and micropropagated plants provides valuable information regarding the maintenance of medicinal properties during in vitro regeneration.

3.1 Phytochemical Profile of Wild and *In Vitro* Propagated Plants

Table 1: Qualitative Phytochemical Screening of Wild and In Vitro Propagated *Lavandula angustifolia* Extracts

Plant Source	Plant Part	Phenolics	Flavonoids	Alkaloids	Terpenoids
Wild	Leaves	+	+	+	+
Wild	Flowers	+	+	+	+
Wild	Stems	+	+	+	+
Wild	Roots	+	+	+	+
In vitro	Leaves	+	+	+	+
In vitro	Flowers	+	+	+	+
In vitro	Stems	+	+	+	+
In vitro	Roots	+	+	+	+

Qualitative phytochemical analysis revealed the presence of important secondary metabolites including phenolic compounds, flavonoids, alkaloids, and terpenoids in different extracts of wild and in vitro propagated *Lavandula angustifolia*. The occurrence of these phytoconstituents indicates the medicinal potential of lavender extracts. Phenolic compounds were detected in different plant parts of both wild and in vitro propagated plants. The presence of phenolics is considered significant because these compounds contribute to antioxidant activity through free radical scavenging mechanisms. Phenolic hydroxyl groups can donate electrons or hydrogen atoms, thereby neutralizing reactive oxygen species. Flavonoids were also detected in the investigated extracts. Flavonoids are important bioactive molecules associated with antioxidant, antimicrobial, and anti-inflammatory activities. Their presence supports the therapeutic importance of *L. angustifolia* and explains its traditional medicinal applications. Terpenoids were detected in different extracts, particularly because lavender is an aromatic plant rich in essential oil components. Terpenoid compounds such as linalool and linalyl acetate are responsible for fragrance as well as antimicrobial properties of lavender essential oil.

The comparable phytochemical profile observed in wild and in vitro propagated plants indicates that tissue culture conditions successfully maintained the biosynthetic ability of regenerated plants. Similar

production of secondary metabolites in micropropagated plants is important for sustainable cultivation and conservation of medicinal plant resources.

3.2 Total Phenolic and Flavonoid Content

Quantitative estimation revealed variation in total phenolic and flavonoid content among different plant parts and solvent extracts of wild and in vitro propagated *L. angustifolia*. The results demonstrated that extraction solvent significantly influenced the recovery of bioactive compounds. Among the tested solvents, ethanol extracts showed comparatively higher phenolic and flavonoid contents than aqueous, chloroform, and petroleum ether extracts. This may be attributed to the higher polarity of ethanol, which facilitates efficient extraction of polar phenolic compounds and flavonoids.

The higher concentration of phenolic compounds observed in ethanol extracts may contribute to enhanced antioxidant activity because phenolics act as efficient free radical scavengers. Similarly, increased flavonoid content may contribute to antioxidant and antimicrobial properties by interacting with cellular targets and oxidative pathways.

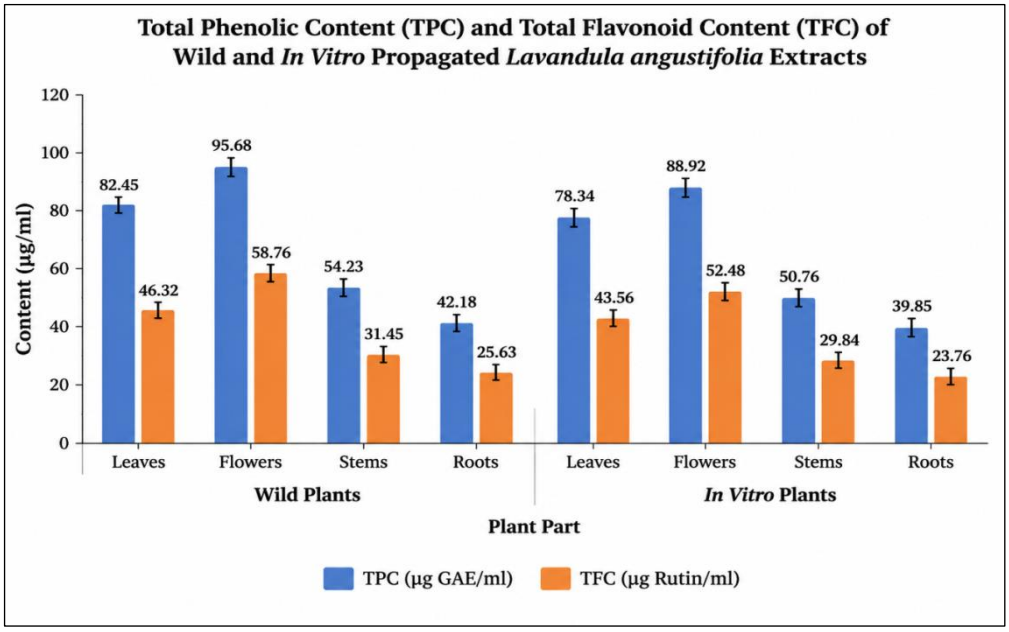

Figure 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Wild and *In Vitro* Propagated *Lavandula angustifolia* Extracts

The comparison between wild and *in vitro* propagated plants showed that regenerated plants retained appreciable levels of phenolic and flavonoid compounds. This indicates that micropropagation did not significantly affect the production of important secondary metabolites and supports the use of tissue culture-derived plants for medicinal applications.

Previous studies have reported that phenolic and flavonoid compounds are major contributors to antioxidant activity in lavender species (Spiridon *et al*., 2011; Prusinowska & Śmigielski, 2014).

3.3 Antioxidant Activity

DPPH Radical Scavenging Activity

The antioxidant potential of wild and in vitro propagated *L. angustifolia* extracts was evaluated using DPPH radical scavenging assay. The extracts exhibited concentration-dependent antioxidant activity, indicating their ability to neutralize free radicals.

Variation in antioxidant activity was observed among different plant parts and extracts. The highest DPPH scavenging activity was recorded in ethanol extracts, which showed a positive correlation with their higher phenolic and flavonoid contents. This confirms that phenolic compounds and flavonoids play an important role in antioxidant defense mechanisms.

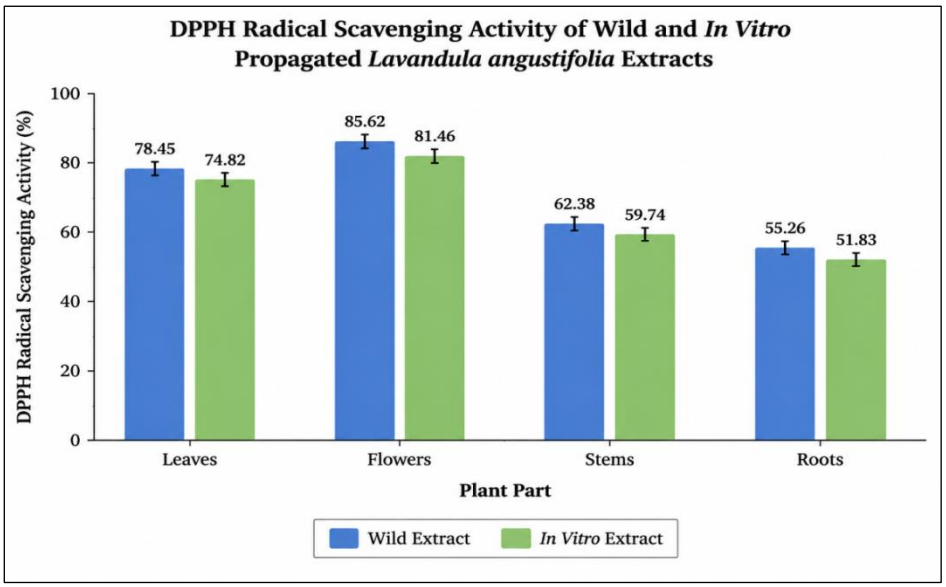

Figure 2: DPPH Radical Scavenging Activity of Wild and *In Vitro* Propagated *Lavandula angustifolia*

Flower and leaf extracts showed comparatively higher antioxidant activity than stem and root extracts, which may be associated with greater accumulation of secondary metabolites in metabolically active aerial tissues.

In vitro propagated plants exhibited antioxidant activity comparable to wild plants, demonstrating that regenerated plants maintained their biochemical potential. This finding is significant because it confirms that micropropagation can be used for large-scale production of medicinally valuable lavender plants without considerable loss of antioxidant properties.

The antioxidant activity observed in the present study supports earlier reports that lavender extracts rich in phenolic compounds possess significant free radical scavenging potential (Spiridon *et al*., 2011).

3.4 Antibacterial Activity Against *Escherichia coli*

The antibacterial activity of wild and in vitro propagated *Lavandula angustifolia* extracts was evaluated against *Escherichia coli* by measuring the zone of inhibition. Considerable variation was observed among different plant parts and solvent extracts. Among all extracts, ethanol extracts exhibited maximum antibacterial activity, followed by chloroform, petroleum ether, and aqueous extracts. In wild plants, the highest antibacterial activity was recorded in flower ethanol extract (18 mm), followed by leaf ethanol extract (17 mm). In comparison, in vitro propagated plants also showed considerable antibacterial potential, with maximum activity observed in flower ethanol extract (16 mm). The superior activity of ethanol extracts may be attributed to efficient extraction of phenolic compounds, flavonoids, and other antimicrobial phytoconstituents. Flowers and leaves showed higher antibacterial activity compared with stems and roots due to greater accumulation of secondary metabolites in metabolically active tissues.

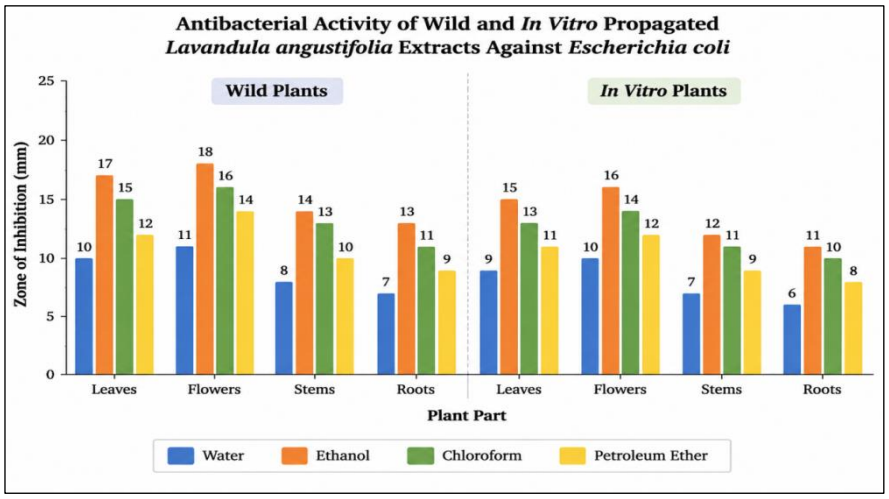

Figure 3: Antibacterial Activity (Zone of Inhibition in mm) of Wild and *In Vitro* Propagated *Lavandula angustifolia* Extracts against *E. coli*

Although wild plants exhibited slightly higher inhibition zones, in vitro propagated plants retained appreciable antibacterial activity, indicating maintenance of bioactive potential during micropropagation.

3.5 Antifungal Activity Against *Aspergillus niger*

The antifungal activity of *L. angustifolia* extracts was assessed against *Aspergillus niger*. All extracts showed variable levels of fungal growth inhibition depending on plant part and solvent used. The highest antifungal activity was observed in ethanol extracts, whereas aqueous extracts showed comparatively lower activity. Among different plant parts, flower extracts exhibited maximum antifungal activity, with wild flower ethanol extract showing the highest zone of inhibition (20 mm), followed by leaf ethanol extract (19 mm). The higher antifungal potential of ethanol extracts may be associated with the presence of phenolic compounds, flavonoids, and terpenoid constituents that interfere with fungal growth and cellular processes. The results indicate that solvent polarity significantly influenced extraction of antifungal compounds. In vitro propagated plants showed comparable antifungal activity, with maximum inhibition recorded in flower ethanol extract (18 mm).

This suggests that regenerated plants retained important bioactive compounds responsible for antifungal activity.

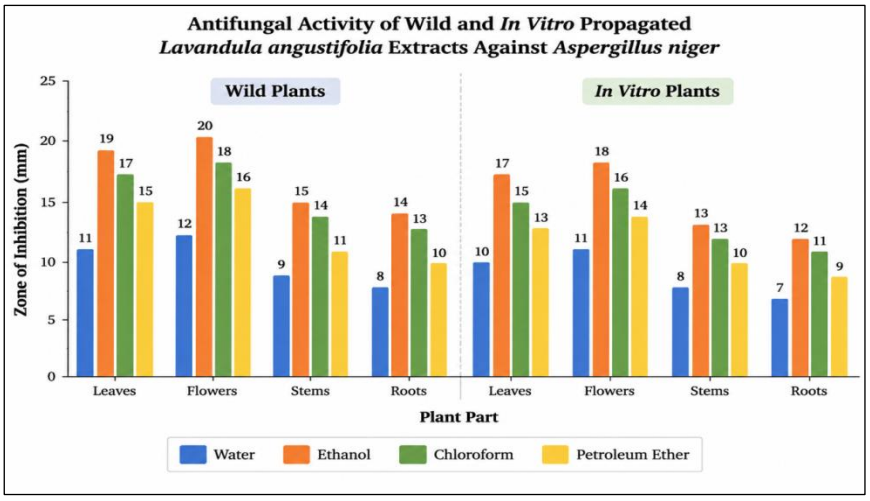

Figure 4: Antifungal Activity (Zone of Inhibition in mm) of Wild and *In Vitro* Propagated *Lavandula angustifolia* Extracts Against *Aspergillus niger*

3.6 Comparative Evaluation of Wild and In Vitro Propagated Plants

The comparative analysis revealed that both wild and in vitro propagated *Lavandula angustifolia* plants possessed significant antioxidant and antimicrobial potential. Although wild plants generally showed slightly higher biological activity, the difference between wild and in vitro plants was comparatively low, indicating successful maintenance of medicinal properties during tissue culture propagation. The comparable phytochemical profile and biological activity of in vitro propagated plants demonstrate that micropropagation does not significantly alter the biosynthetic capacity of *L. angustifolia*. The presence of phenolics, flavonoids, and terpenoid compounds in regenerated plants supports their antioxidant and antimicrobial potential. The successful establishment of in vitro propagated plants provides an effective alternative for large-scale production and conservation of this valuable medicinal plant. Micropropagation can help overcome limitations associated with slow conventional multiplication and excessive harvesting of natural populations. Overall, the findings suggest that in vitro propagated *L. angustifolia* plants can serve as a reliable source of bioactive compounds and may be utilized for sustainable production of medicinally important plant material.

4. CONCLUSION

The present study demonstrated that wild and in vitro propagated *Lavandula angustifolia* Mill. exhibited considerable antioxidant and antimicrobial potential. The biological activities varied among different plant parts and solvent extracts, with ethanol extracts showing superior performance, which may be associated with efficient extraction of phenolic compounds, flavonoids, and other bioactive constituents. The antimicrobial evaluation revealed significant inhibitory activity against *Escherichia coli* and *Aspergillus niger*, particularly in flower and leaf extracts. In vitro propagated plants showed comparable phytochemical composition and biological activity to wild plants, indicating successful maintenance of medicinal properties during micropropagation. These findings highlight the potential of *L. angustifolia* as a valuable source of natural antioxidant and antimicrobial agents. Furthermore, in vitro propagation offers an effective and sustainable approach for large-scale multiplication, conservation, and continuous availability of medicinally important plant material. Thus, the integration of tissue culture technology with phytochemical and biological evaluation can support the sustainable utilization of *Lavandula angustifolia* for pharmaceutical and biotechnological applications.

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