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In Vitro Propagation as a Strategy for Improving Phytochemical Production and Supporting Conservation of Tunisian *Lavandula stoechas*

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Highlights

What are the main findings?

- An efficient in vitro propagation protocol was developed for Tunisian *Lavandula stoechas* starting from seeds, with optimal shoot biomass and multiplication at 3 µM BAP.
- In vitro cultured plants exhibited fivefold higher total phenolic content and significantly higher rosmarinic acid levels than wild-growing plants, along with superior antioxidant activity.

What are the implications of the main findings?

- This micropropagation system offers an effective strategy for large-scale production of bioactive metabolites in *L. stoechas*.
- It also supports both commercial exploitation and future conservation efforts for this species.

Abstract

Lavandula stoechas (Lamiaceae) is a highly valued medicinal plant due to its rich content of bioactive secondary metabolites, particularly rosmarinic acid. This study aimed to develop an efficient in vitro propagation protocol from seeds and to evaluate the influence of 6-benzylaminopurine (BAP) on morphogenesis and phenolic compound production while comparing the antioxidant and antifungal activities of methanolic extracts from in vitro cultured and wild-growing plants. Seeds were germinated on half-strength MS medium under different sucrose concentrations and temperatures. Nodal explants from seedlings were cultured on full-strength MS medium supplemented with 0, 1, 2, or 3 µM BAP. Methanolic extracts were analyzed for total phenolic content, rosmarinic acid concentration (HPLC), antioxidant activity (DPPH and ABTS assays), and antifungal activity

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against phytopathogenic fungi. Rapid germination occurred on day 3 on half-strength MS medium with 1% sucrose at 20 °C. The highest biomass production was achieved with 3 µM BAP. In vitro cultured plants accumulated fivefold-higher total phenolic content (217.73 mg GAE g^{-1} DW) than wild plants (43.23 mg GAE g^{-1} DW), with significantly higher rosmarinic acid levels. Antioxidant activity was markedly stronger in extracts from plants grown with 1–2 µM BAP, whereas antifungal activity remained limited. These results demonstrate that in vitro propagation with optimized BAP concentrations is an effective strategy for enhancing bioactive metabolite production and supporting the conservation of Tunisian *L. stoechas*.

Keywords: micropropagation; phenolic compounds; rosmarinic acid; antioxidant activity; antifungal activity; Tunisian Lavender

1. Introduction

The Lamiaceae family encompasses numerous species of significant global economic importance, valued for their aromatic, medicinal, culinary, and ornamental uses. Within this family, the genus *Lavandula* is particularly prominent, comprising some of the most popular ornamental and medicinal species [1]. In Tunisia, the genus is represented by several key species, most notably *L. stoechas*, *L. multifida*, *L. dentata*, and *L. coronopifolia* [2].

Lavandula stoechas is recognized for its substantial accumulation of essential oils and other bioactive secondary metabolites, particularly phenolic compounds. These metabolites contribute to the plant's well-documented hypnotic properties [3], antibacterial and antileishmanial activities [4], and remarkable efficacy in treating sinusitis [5]. Previous studies have revealed that *L. stoechas* extracts exhibit antioxidant effects [6,7]. Phenolic compounds are considered largely responsible for its therapeutic properties. Gilani et al. [8] showed that its aqueous–methanolic extract possesses anticonvulsant and antispasmodic activities. Significant antimicrobial activity has also been reported for ethanolic extracts [9,10]. In addition, Kulabas et al. [11] found that aqueous extracts were effective in preventing insulin resistance and inflammation.

To meet growing pharmaceutical and industrial demands, the economic exploitation of this plant has intensified in its natural habitats, posing a serious threat to wild populations. Moreover, our recent study revealed a low level of genetic diversity in Tunisian natural populations of *L. stoechas* compared to other threatened wild species [12]. Furthermore, *L. stoechas* produces very small seeds, with a thousand-grain weight of less than 1 g [13], which results in slow and poor germination, typically below 10% [14].

Several studies on germination methods have evaluated the effects of different treatments on the germination capacity of *L. stoechas* seeds. In vitro germination of *L. stoechas* seeds, as in other plant species, can be influenced by culture conditions (e.g., temperature), the composition of the culture medium (e.g., sucrose concentration), and the particular characteristics of the seeds (e.g., dormancy) [15]. To our knowledge, the combined effects of temperature and sucrose concentration on the in vitro germination of *L. stoechas* seeds have not been documented.

Furthermore, it is well known that in vitro conditions largely affect the production of secondary metabolites and, consequently, their biological activity; hence, it is important to develop in vitro propagation methods for the rapid and efficient production of high-value phytochemical compounds [16]. Among these compounds, rosmarinic acid (RA) occupies a prominent position due to its remarkable bioactive properties. RA is a natural antioxidant widely distributed within the Lamiaceae family [17]. In recent years, RA has

attracted increasing interest because of its diverse biological activities, as this compound exhibits cardioprotective, nephroprotective, anti-aging, and antidepressant effects [18–21]. Moreover, it shows significant anticancer activity by modulating tumor cell proliferation, apoptosis, and inflammation [22].

According to the literature, only a few studies have reported in vitro propagation of *L. stoechas* [23–25]. These previous attempts mainly focused on shoot multiplication using limited plant growth regulator (PGR) regimes and provided only partial data on secondary metabolites. Importantly, none of them evaluated the biological activities (e.g., antifungal properties) of extracts from micropropagated plants in comparison with wild-growing material. The present study was therefore undertaken to address these gaps by evaluating the effects of in vitro culture conditions and PGRs on secondary metabolite accumulation and the biological activity of micropropagated *L. stoechas* plants, as part of a broader ongoing research project on the conservation of Tunisian lavender germplasm. We focused primarily on the effects of in vitro culture and the cytokinin 6-benzylaminopurine (BAP) on the production of different classes of phenolic compounds, as well as on the biological activity of micropropagated *L. stoechas*. Finally, we compared, for the first time, the antifungal activities of *L. stoechas* methanolic extracts against phytopathogenic fungi in wild-growing versus in vitro plants. The obtained results provide a valuable contribution to the conservation of the species and its commercial exploitation for the isolation of valuable bioactive compounds without threatening the biodiversity of natural ecosystems.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *L. stoechas* were collected from its natural habitat in El Feija, north-western Tunisia (36°29′10″ N, 8°18′34″ E). Harvesting was performed once, during the late flowering stage in July 2023. The collected samples were air-dried in the shade at room temperature. Healthy, intact, and mature seeds (brownish–black in color) were manually extracted in the laboratory and stored in amber glass bottles at 4 °C in a refrigerator until further use. The remaining dried aerial parts were finely ground and stored at 4 °C for subsequent bioactivity assays.

2.2. Seed Germination

The seeds were rinsed with sterile distilled water and then surface-sterilized by immersion in 70% (*v*/*v*) ethanol for 50 s, followed by immersion in 10% (*v*/*v*) commercial bleach (containing 4% (*w*/*v*) active chlorine) for 10 min. After disinfection, seeds were rinsed three times with sterile distilled water. Surface-sterilized seeds were inoculated into Petri dishes containing half-strength MS (½MS) medium [26], supplemented with 7 g L^{-1} agar (Torlak, Belgrade, Serbia) and sucrose at concentrations of either 10 g L^{-1} (1%) or 20 g L^{-1} (2%). The pH of the medium was adjusted to 5.8 before autoclaving at 114 °C for 25 min. For each sucrose concentration, three replicates of ten seeds were used per temperature treatment, and the experiment was repeated, resulting in a total of 60 seeds per treatment. Cultures were incubated at either 20 °C or 25 °C, all maintained under a 16 h light/8 h dark photoperiod with a photon flux density of 45 µmol m^{-2} s^{-1}. Germination was monitored daily for 30 days and was considered to have occurred when radicle length reached at least 2 mm.

2.3. Micropropagation

Nodal segments from 4-week-old seedlings were used as explants to establish shoot cultures. The nodal segments were excised and placed on a full-strength basal MS medium supplemented with 3% (*w*/*v*) sucrose and 0.7% (*w*/*v*) agar in 250 mL glass jars containing

25 mL of medium. Ten explants were placed in each jar. Cultures were maintained in a growth chamber equipped with LED FARM series LED modules (5000 K; Center of LED and Optoelectronic Technologies of the National Academy of Sciences of Belarus, Minsk, Belarus) under a 16 h photoperiod at a photosynthetic photon flux density (PPFD) of 45 μmol m^{-2} s^{-1} and 25 ± 2 °C. Routine subculturing was performed at 6-week intervals. Healthy nodal segments (approximately 1 cm in length, containing one node) were excised from vigorously growing shoots. Any jars showing contamination were discarded entirely, immediately upon detection.

To investigate the effects of BAP on morphogenesis and the production of secondary metabolites, nodal segments derived from 4-week-old seedlings were transferred to a basal MS medium supplemented with BAP at different concentrations: 0, 1, 2, and 3 μM. Three biological replicates, each consisting of ten explants per jar, were used per treatment, and the experiment was repeated once, resulting in a total of 60 explants per treatment. The cultures were maintained in a growth chamber under the same growth conditions as those used for shoot culture establishment. After 4 weeks, the fresh and dry weight of the explants, the number of shoots per explant, shoot length, and the number of leaves were recorded in order to evaluate the effect of different BAP concentrations on shoot multiplication.

2.4. Acclimatization

Only plantlets maintained on PGR-free medium were used for acclimatization experiment. After 4 weeks of cultivation on the basal MS medium without PGRs, the spontaneously rooted plantlets appeared healthy, with well-developed shoots and highly branched adventitious roots. A total of 51 whole plantlets were gently removed from the culture vessels, and the roots and basal stem parts were carefully washed with tap water to remove residual culture medium. The plantlets were then transferred to plastic pots (approximately 750 mL volume; 2–4 plantlets per pot) containing a commercial substrate ("Substral"; Scotts Poland Sp. z o.o., Warszawa, Poland). The pots were covered with thin, transparent plastic bags to reduce water loss and maintained in a greenhouse under high relative humidity (70–80%). Gradual acclimatization was performed over a 4-week period by progressively increasing the daily ventilation time until the plastic covers were completely removed. Plant survival was evaluated after 8 weeks.

2.5. Preparation of Methanolic Extracts

The collected plant samples were air-dried at room temperature and ground to a fine powder using a mortar and pestle. A 500 mg portion of the powder obtained from both wild-growing and in vitro regenerated plants was extracted with 10 mL of methanol in an ultrasonic bath (Bandelin Sonorex RK 100, Berlin, Germany) operating at a frequency of 35 Hz and a power of 80 W at room temperature for 20 min. After sonication, the extracts were filtered through 0.45-μm nylon syringe filters (Captiva Econo Filters, 13 mm; Agilent Technologies, Santa Clara, CA, USA) and stored at 4 °C until further analysis. All subsequent biochemical and biological activity assays were performed in biological triplicates (n = 3).

2.6. Determination of Total Bioactive Compounds

2.6.1. Determination of Total Phenolic Content

The total phenolic content was estimated using the Folin–Ciocalteu method [27]. The diluted extract (100 μL) was mixed with 500 μL of Folin–Ciocalteu reagent (diluted 10 times with distilled water). After a 5 min incubation, 400 μL of 7.5% (w/v) sodium carbonate was added. After incubating for 90 min, the absorbance was measured at 760 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAEs) per gram of dry weight (mg GAE g^{-1} DW).

2.6.2. Determination of Total Flavonoid Content

The total flavonoid content was determined according to the method described by Djeridane et al. [28]. Briefly, a 500 µL aliquot of 2% (*w*/*v*) aluminum chloride (in methanol) was added to 500 µL of the diluted plant extract. After incubation at room temperature for 15 min, the absorbance was measured at 430 nm. The total flavonoid content was expressed as milligrams of rutin equivalents (REs) per gram of dry weight (mg RE g^{-1} DW).

2.6.3. Determination of Total Flavonol Content

The total flavonol content in the plant extracts was estimated using the method of Kumaran and Karunakaran [29]. A 2 mL aliquot of the diluted extract was mixed with an equal volume of a 2% (*w*/*v*) $AlCl_3$ ethanolic solution and 3 mL of a 50 g L^{-1} sodium acetate solution. The mixture was incubated at 20 °C for 2.5 h, and the absorbance was measured at 440 nm. The total flavonol content was expressed as milligrams of quercetin equivalents (QEs) per gram of dry weight (mg QE g^{-1} DW).

2.6.4. Determination of Total Condensed Tannin Content

The condensed tannin content was measured according to the protocol described by Oyedemi et al. [30]. To a 100 µL aliquot of the diluted extract, 600 µL of a 4% (*w*/*v*) vanillin solution (in methanol) and 300 µL of concentrated hydrochloric acid were added. After incubation for 15 min at room temperature, the absorbance was measured at 500 nm. The total condensed tannin content was expressed as milligrams of catechin equivalents (CEs) per gram of dry weight (mg CE g^{-1} DW).

2.7. Determination of Phenolic Acids by HPLC

The identification and quantification of phenolic acids were performed by chromatographic analysis using an Agilent 1100 Series HPLC instrument equipped with a diode array detector and a reversed-phase Zorbax SB-C18 (Agilent) analytical column (250 mm × 4.6 mm i.d., 5 µm particle size) thermostated at 25 °C. The mobile phase consisted of solvent A (0.1% (*v*/*v*) ortho-phosphoric acid in water) and solvent B (acetonitrile, J.T. Baker, Deventer, The Netherlands). The separation was achieved using the following gradient elution program: 0–10 min, 75–73% A; 10–20 min, 73–65% A; 20–25 min, 65–30% A; and 25–32 min, 30–0% A. The flow rate was 0.9 mL min^{-1}, the sample injection volume was 10 µL, and the column elution was monitored at 280 and 330 nm.

Rosmarinic and caffeic acids were identified by comparison with standard compounds purchased from Sigma-Aldrich (Steinheim, Germany). Quantification was performed using external standard calibration curves. Stock solutions of the standards were prepared by dissolving the compounds in methanol. Linear regression analyses of the calibration curves showed high linearity, with correlation coefficients (r^2) ranging from 0.9996 to 0.9998. The amounts of the analyzed compounds were expressed as mg per g of dry weight (mg g^{-1} DW).

2.8. Evaluation of Antioxidant Activity

The in vitro antioxidant activity of the methanolic extracts obtained from wild-growing and in vitro regenerated plants was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays.

2.8.1. DPPH Assay

The DPPH free radical scavenging activity was determined using the method described by Doshi et al. [31]. Briefly, a 50 µL aliquot of the diluted extract was mixed with 950 µL of a 60 µM DPPH methanolic solution. The mixture was vortexed and incubated for 30 min in the dark at room temperature. The absorbance was recorded at 517 nm. A mixture consisting of 50 µL of methanol and 950 µL of the DPPH solution served as the control.

All experiments were performed in triplicate. The results were expressed as milligrams of Trolox equivalents (TEs) per gram of dry weight (mg TE g^{-1} DW).

2.8.2. ABTS Assay

The ABTS radical scavenging assay was carried out according to the method of Re et al. [32] with slight modifications. Briefly, a 10 µL aliquot of each extract was vigorously mixed with 990 µL of the diluted ABTS radical cation solution. The mixture was incubated for 20 min in the dark at room temperature, and the absorbance was recorded at 734 nm. A mixture of 10 µL of methanol and 990 µL of the diluted ABTS solution was used as the control. Each determination was carried out in triplicate, and the results were expressed as milligrams of Trolox equivalents per gram of dry weight (mg TE g^{-1} DW).

2.9. Antifungal Activity Assay

The methanolic extracts were dried using a rotary evaporator and then re-dissolved in DMSO. Briefly, the antifungal activity of the extracts was evaluated using the disc diffusion method [33], and the fungal mycelium growth was studied in Petri dishes on PDA medium. Sterile filter paper discs (6 mm in diameter) impregnated with 25 µL of each methanolic extract were placed on the surface of the medium. The Petri dishes were then sealed with Parafilm and incubated at 25 °C. The zones of inhibition were measured and expressed as diameters in millimeters (mm). The antifungal activity was assessed based on the growth rate of the fungal species. DMSO was used as the negative control. No positive reference fungicides were included in the assay, as the objective was to compare the relative activity of extracts from wild-growing versus in vitro propagated plants rather than to evaluate them against commercial standards. The analyses were performed in triplicate using fungi from the fungal culture collection of the Laboratory of Management and Valorization of Forest Resources at the National Research Institute for Rural Engineering, Water and Forests (INRGREF).

2.10. Statistical Analysis

For the statistical analysis of the germination data, the germination percentages were arcsine-transformed before analysis. All morphological, biochemical and biological activity data were subjected to one-way analysis of variance (ANOVA) using Statgraphics Centurion XV.I software (version 15.01.0002; StatPoint Technologies, Warrenton, VA, USA). Significant differences between treatment means were determined using Duncan's multiple range test at a significance level of $p \leq 0.05$. Different letters within the same column (tables) or above bars (figures) indicate statistically significant differences.

Biological replication details are as follows: three biological replicates (each with 10 seeds per Petri dish or 10 explants per jar) were used per treatment for germination and micropropagation experiments, with the entire experiment repeated once (total $n = 60$ per treatment). All biochemical and biological activity assays were performed in biological triplicate ($n = 3$). Data are expressed as mean ± standard error (SE).

3. Results

3.1. Seed Germination

The germination percentage of the seeds in vitro was affected by the combination of temperature and sucrose concentration in the ½MS medium, as shown in Figure 1. As expected, germination rates were higher at 1% sucrose than at 2% sucrose at both temperatures, although the differences in final germination percentages between the two sucrose concentrations within the same temperature were not statistically significant. Furthermore, temperature exerted a significant effect on the final results, with seeds cultivated at 20 °C

achieving a significantly higher final germination percentage than those at 25 °C, regardless of sucrose concentration.

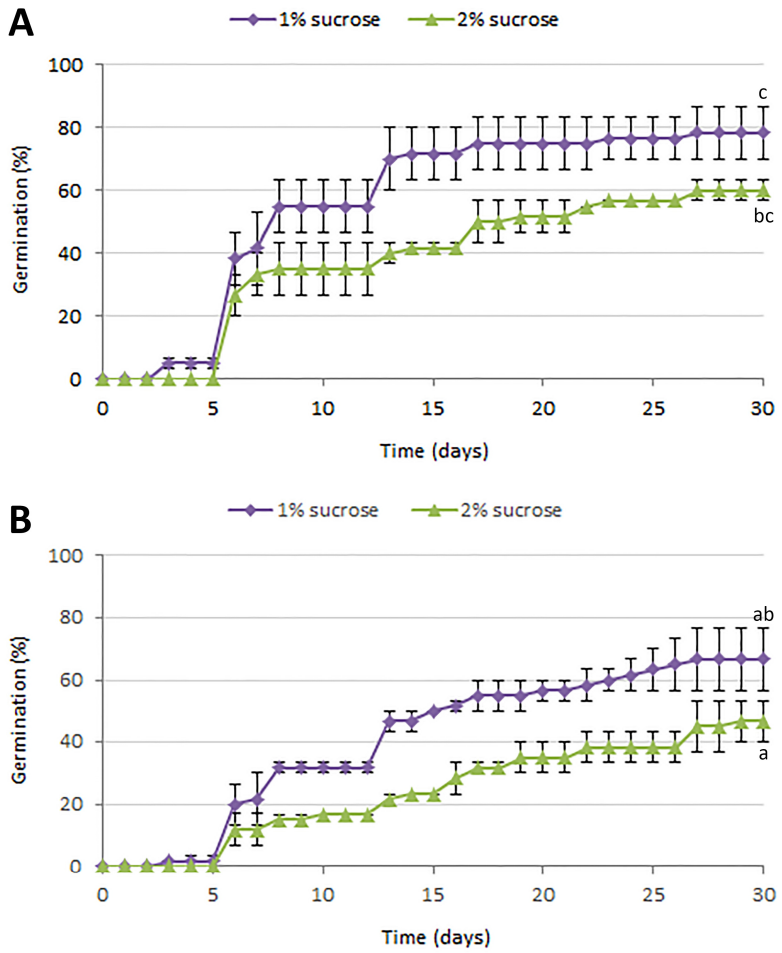

Figure 1. Time course of cumulative germination percentage of *Lavandula stoechas* seeds as affected by sucrose concentration (1% and 2%) and temperature: 20 °C (**A**) and 25 °C (**B**). Data are presented as mean ± standard error (SE). Different lowercase letters at Day 30 indicate significant differences among treatments at the end of the experiment according to Duncan's multiple range test ($p \leq 0.05$).

At a temperature of 20 °C (Figure 1A), the first germinated seeds were observed on day 3 after sowing on the ½MS medium supplemented with 1% sucrose. On day 6, the germination percentage on this medium reached 38%. On medium with 2% sucrose, the appearance of radicles was first recorded on day 6 after sowing, with a germination percentage of 27%. After 27 days, the seed germination percentage was 78% at the lower sucrose concentration (1%) and 60% at the higher concentration (2%). At a temperature of 25 °C (Figure 1B), regardless of the sucrose content, germination commenced on day 6, with a percentage of around 20% for the seeds germinated on the ½MS medium with 1% sucrose and 12% for the medium with the higher sucrose concentration (2%). The germination percentages reached a plateau towards the end of the experiment, with 67% on the medium with 1% sucrose and 47% on the medium with 2% sucrose.

3.2. Micropropagation

Shoot cultures of *L. stoechas* were successfully established from nodal segments excised from 4-week-old in vitro grown seedlings derived from seeds of wild-growing plants (Figure 2A,B). Axillary shoots developed from each explant after 4 weeks of cultivation on the basal MS medium without PGRs (Figure 2C).

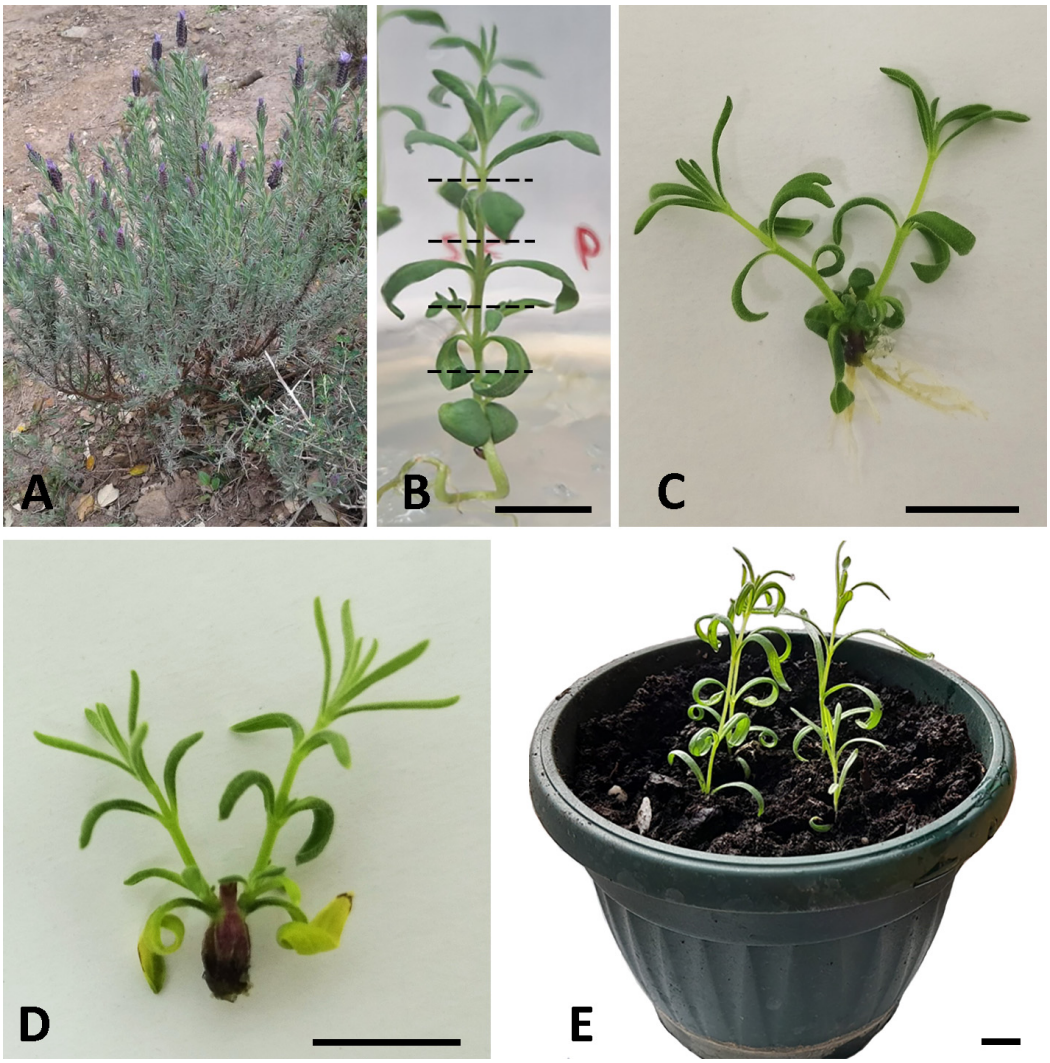

Figure 2. Micropropagation of *Lavandula stoechas*. (**A**) Wild-growing plant in its natural habitat (average plant height: 60–100 cm); (**B**) A 4-week-old in vitro seedling; dashed lines indicate the excision sites for obtaining single-node stem segments; (**C**) An in vitro plantlet cultured on a PGR-free medium; (**D**) Axillary shoot proliferation on a culture medium supplemented with 3 µM BAP; (**E**) *L. stoechas* plants produced using the established micropropagation protocol after 2 months of acclimatization. Scale bars = 1 cm.

The effects of BAP on the growth parameters of *L. stoechas* were recorded after 28 days of cultivation (Table 1). BAP significantly affected the number of shoots per explant, which increased with increasing BAP concentration and reached its maximum (8.44 ± 0.37) at 3 µM BAP. The highest shoot length was obtained on BAP-free medium and did not differ significantly from most BAP treatments, except for 2 µM BAP (Table 1). The number of leaves per explant was higher in all BAP-treated plants compared to the PGR-free control, with the maximum observed on medium supplemented with 3 µM BAP (Figure 2D). Morphologically, plantlets on PGR-free medium were characterized by elongated shoots with longer internodes and fewer axillary buds, resulting in a less compact appearance. In contrast, BAP supplementation induced compact shoots with shortened internodes and increased axillary branching. This effect was most pronounced at 3 µM BAP, producing denser and more compact multiple shoots. Compared to the PGR-free medium, BAP at all tested concentrations increased biomass production. The maximum fresh and dry weights per explant were obtained on the medium supplemented with 3 µM BAP, representing an

increase of more than 70% in both parameters (Table 1). All media tested induced callus formation at the basal cut end of the explants, especially at the highest BAP concentration, which produced a compact, green callus mass.

Table 1. Effect of different BAP concentrations on the morphogenic responses of *Lavandula stoechas* explants after 28 days of culture.

BAP (µM)	Explant Fresh Weight (mg)	Explant Dry Weight (mg)	Number of Shoots per Explant	Shoot Length (mm)	Number of Leaves per Explant
0	102.77 ± 8.02 a	13.76 ± 0.68 a	3.49 ± 0.30 a	**14.81 ± 1.20 b**	20.52 ± 1.00 a
1	148.14 ± 10.82 b	18.86 ± 0.89 b	4.53 ± 0.31 b	12.96 ± 0.50 ab	24.48 ± 1.02 b
2	145.08 ± 11.79 b	18.65 ± 1.16 b	5.79 ± 0.41 c	12.11 ± 0.57 a	26.72 ± 1.57 b
3	**183.50 ± 10.19 c**	**24.01 ± 0.96 c**	**8.44 ± 0.37 d**	**14.77 ± 0.51 b**	**37.15 ± 1.26 c**

Values are means ± SE, n = 60. Means within a column followed by different letters are significantly different according to Duncan's multiple range test ($p \leq 0.05$). Maximum values are in bold.

3.3. Rooting and Acclimatization

Following culture initiation, the explants grew vigorously. Spontaneous root formation was observed on PGR-free medium with an 85% rooting frequency. Upon transfer to greenhouse conditions, 89% of the rooted plantlets survived acclimatization. The high spontaneous rooting frequency, combined with the excellent acclimatization survival rate and the development of vigorous root systems, demonstrates effective rooting performance of the established protocol. The acclimatized plants appeared morphologically normal and healthy, with no visible abnormalities (Figure 2E).

3.4. Determination of Phenolic Compounds

3.4.1. Total Bioactive Compounds Content

The phenolic compound contents in the methanolic extracts obtained from wild-growing and micropropagated plants of *L. stoechas* are presented in Table 2. Our results revealed significant differences between the wild-growing and in vitro cultured plants. The wild-growing plants exhibited the lowest total phenolic content (43.23 mg GAE g^{-1} DW) and flavonol content (3.71 mg QE g^{-1} DW). In vitro culture considerably improved the production of these compounds. The total polyphenol content on the PGR-free medium (217.73 mg GAE g^{-1} DW) was fivefold higher than in the wild-growing plants. A significant increase in the total phenolic content, ranging from two- to fourfold of that obtained for the plants from nature, was observed for all BAP-supplemented media (Table 2). The contents of flavonoids (72.37 mg RE g^{-1} DW), flavonols (14.17 mg QE g^{-1} DW), and condensed tannins (3.32 mg CE g^{-1} DW) were also the highest on the PGR-free medium. An increase in flavonoid content was further observed in the plants cultured at lower BAP concentrations, compared to the wild-growing plants. In addition, BAP at all tested concentrations exerted a stimulatory effect on flavonol production, compared to the wild-growing plants (Table 2). However, the plant extracts from the BAP-supplemented media yielded the lowest condensed tannin contents, regardless of the BAP concentration.

Table 2. Total phenolic, flavonoid, flavonol, and condensed tannin contents of the methanolic extracts obtained from the aerial parts of *Lavandula stoechas* wild-growing plants and those cultured in vitro on MS medium supplemented with BAP at different concentrations (0, 1, 2, and 3 µM).

Extracts	Total Phenolics (mg GAE g^{-1} DW)	Flavonoids (mg RE g^{-1} DW)	Flavonols (mg QE g^{-1} DW)	Condensed Tannins (mg CE g^{-1} DW)
Wild-growing plants	43.23 ± 0.94 a	27.80 ± 0.82 a	3.71 ± 0.02 a	1.92 ± 0.03 b

Table 2. *Cont.*

Extracts		Total Phenolics (mg GAE g^{-1} DW)	Flavonoids (mg RE g^{-1} DW)	Flavonols (mg QE g^{-1} DW)	Condensed Tannins (mg CE g^{-1} DW)
In vitro plants	0 µM BAP	**217.73 ± 4.56 e**	**72.37 ± 3.94 c**	**14.17 ± 0.30 d**	**3.32 ± 0.20 c**
	1 µM BAP	176.27 ± 0.42 d	37.37 ± 0.79 b	7.63 ± 0.13 c	0.64 ± 0.02 a
	2 µM BAP	102.43 ± 1.92 b	35.03 ± 0.09 b	7.28 ± 0.14 c	0.78 ± 0.03 a
	3 µM BAP	126.7 ± 1.82 c	24.62 ± 0.40 a	5.39 ± 0.08 b	0.86 ± 0.01 a

Values are means ± SE (n = 3). Different letters within a column indicate statistically significant differences ($p \leq 0.05$) according to Duncan's multiple range test. The maximum values are indicated in bold.

3.4.2. HPLC Analysis of Phenolic Acids

High-performance liquid chromatography (HPLC) was employed to profile and quantify the phenolic acids present in the methanolic extracts of *L. stoechas*. The resulting chromatograms revealed that rosmarinic acid (RA) was the major component detected in all analyzed extracts, followed by caffeic acid (CA) as the second identified peak (Figure 3).

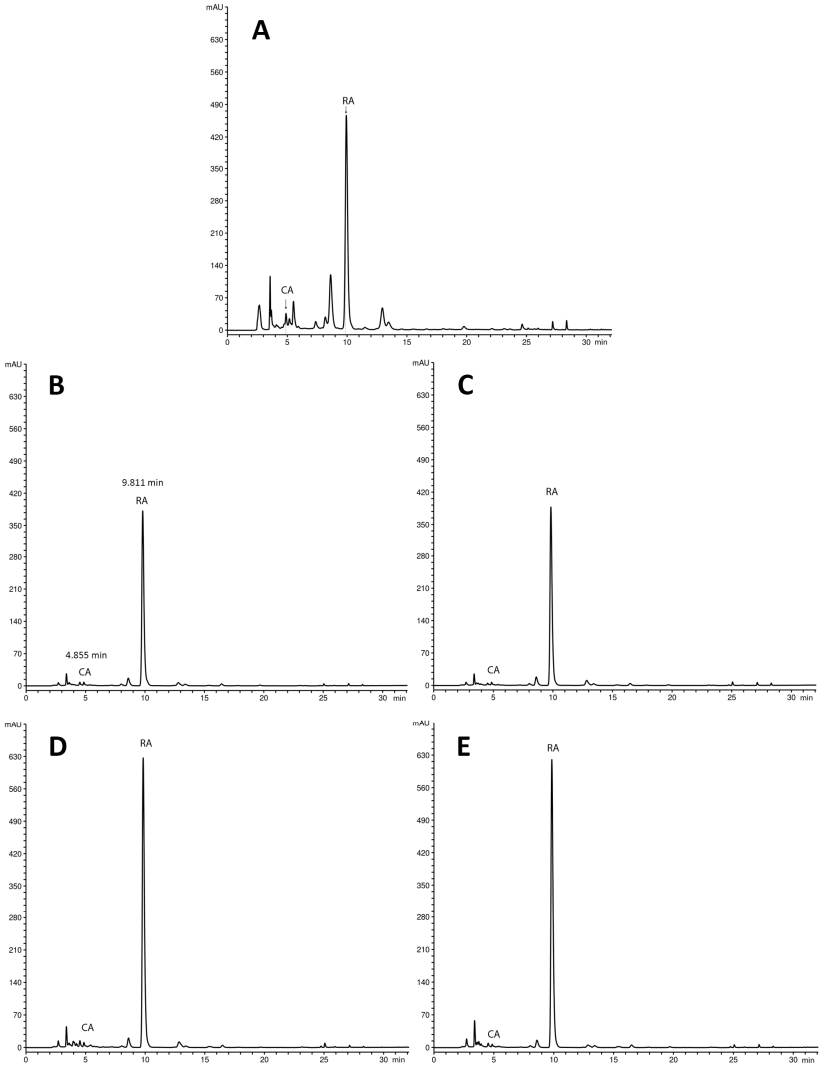

Figure 3. Comparative HPLC chromatograms of the methanolic extracts obtained from wild-growing plants (**A**) and in vitro cultured plants (**B**–**E**) of *Lavandula stoechas.* In vitro plants were cultured on MS medium devoid of PGRs (**B**) or supplemented with BAP at 1 µM (**C**), 2 µM (**D**) or 3 µM (**E**). Identified peaks: caffeic acid (CA; Rt = 4.855 min) and rosmarinic acid (RA; Rt = 9.811 min). Chromatograms were monitored at 330 nm.

Compared to the wild-growing plants, the in vitro cultured plants exhibited a significantly increased RA content (Figure 4). In particular, the accumulation of RA was highest in the extracts derived from plants treated with 2 µM BAP (33.74 mg g^{-1} DW) and 3 µM BAP (33.32 mg g^{-1} DW). Conversely, the highest caffeic acid content (0.339 mg g^{-1} DW) was observed in the extracts obtained from the wild-growing plants (Figure 4).

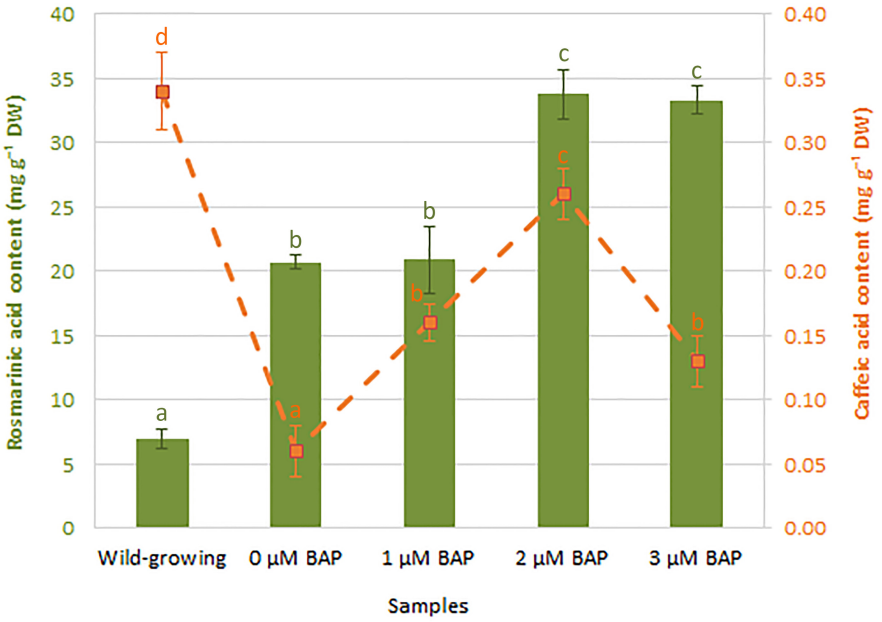

Figure 4. Comparison of rosmarinic acid (RA) and caffeic acid (CA) contents of methanolic extracts across the aerial parts samples of *Lavandula stoechas* obtained from the natural populations (wild-growing) and cultured in vitro on MS medium devoid of PGRs (0 µM) or supplemented with BAP at concentrations of 1, 2, and 3 µM. Values are means ± SE (n = 3). Means with different lower-case letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

3.5. Evaluation of Antioxidant Activity

The antioxidant potential of the methanolic extracts of the aerial parts of the wild-growing and in vitro cultivated *L. stoechas* plants was evaluated using both DPPH and ABTS free radical scavenging assays. All tested extracts showed significant antioxidant activity (Figure 5). The extract obtained from the plants treated with 1 µM BAP proved to be the most effective in scavenging DPPH radicals (172.02 mg TE g^{-1} DW). On the other hand, the lowest antiradical activity (13.27 mg TE g^{-1} DW) was recorded for the wild-growing plants (Figure 5A). The antioxidant activity evaluated by the ABTS assay was higher than that obtained by the DPPH assay for all tested extracts. The highest radical scavenging activity (564.38 mg TE g^{-1} DW) was found in the extracts from the plants treated with 2 µM BAP, while the lowest scavenging capacity (84.53 mg TE g^{-1} DW) was observed in the wild-growing plants (Figure 5B).

The relationships among the individual phenolic compounds and antioxidant activities were evaluated using Pearson's correlation analysis. As shown in Table 3, rosmarinic acid content exhibited a strong positive correlation with both DPPH radical scavenging activity (r = 0.77) and ABTS radical cation scavenging activity (r = 0.84). Total phenolic content was also positively correlated with DPPH (r = 0.73) and ABTS (r = 0.63), supporting the major contribution of these compounds to the antioxidant capacity of *L. stoechas* extracts (Table 3). In contrast, caffeic acid displayed strong negative correlations with these antioxidant assays (DPPH: r = −0.76; ABTS: r = −0.59), while condensed tannins showed weaker, negative correlations with both assays.

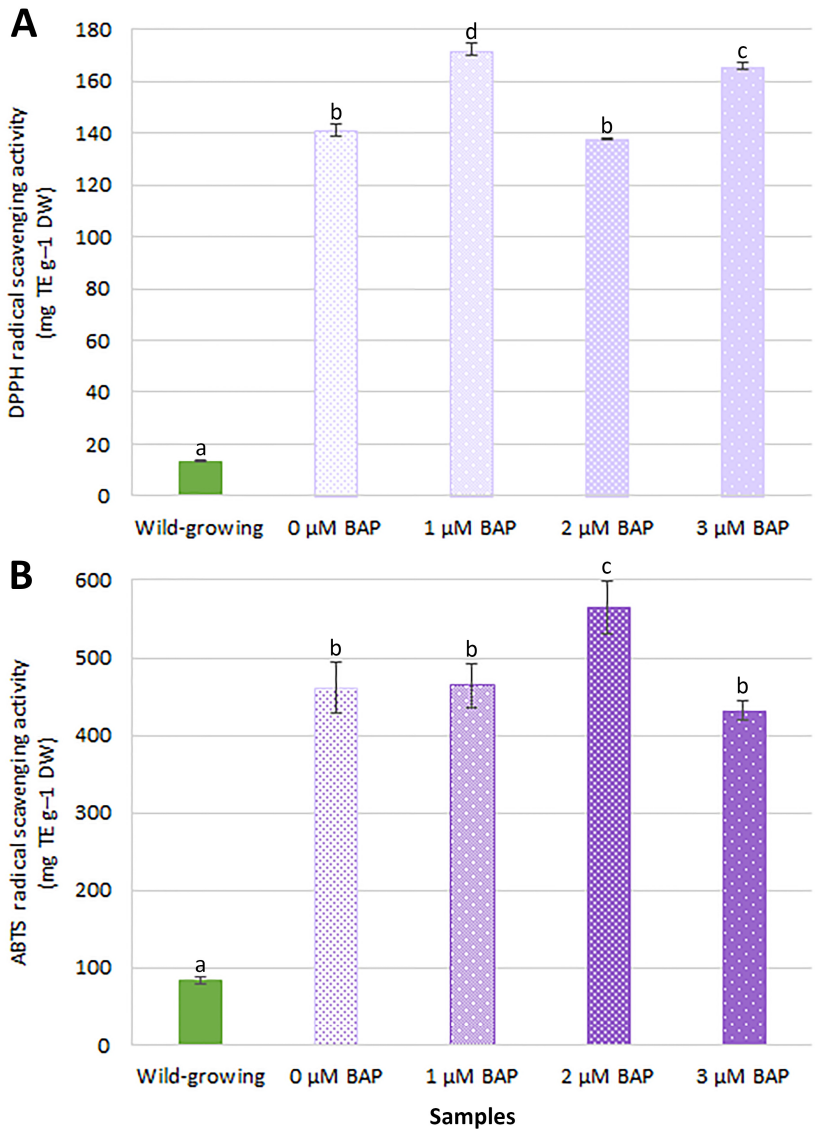

Figure 5. Antioxidant activity of methanolic extracts from wild-growing and in vitro cultured *Lavandula stoechas* plants. Plants were grown on MS medium without PGRs (0 µM BAP) or supplemented with 1, 2, or 3 µM BAP. Activity was assessed using (**A**) DPPH and (**B**) ABTS assays. Values are means ± SE (n = 3). Means with different lower-case letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Table 3. Matrix of Pearson's correlation coefficients (r) among individual phenolic compounds and antioxidant activities in *Lavandula stoechas* extracts (n = 3). Background color intensity represents the strength and direction of the linear relationships (green = positive correlation, red = negative correlation). Statistical significance (p-values) is omitted owing to the pilot sample size (n = 3).

	Total Phenolics	Flavonoids	Flavonols	Condensed Tannins	Rosmarinic Acid	Caffeic Acid	DPPH	ABTS
Total phenolics	1.00							
Flavonoids	0.77	1.00						
Flavonols	0.87	0.97	1.00					
Condensed tannins	0.34	0.80	0.66	1.00				
Rosmarinic acid	0.25	−0.10	0.12	−0.47	1.00			
Caffeic acid	−0.93	−0.64	−0.76	−0.32	−0.39	1.00		
DPPH	0.73	0.22	0.44	−0.32	0.77	−0.76	1.00	
ABTS	0.63	0.31	0.52	−0.26	0.84	−0.59	0.89	1.00

3.6. Antifungal Activity Assay

The antifungal potency of methanolic extracts isolated from wild-growing *L. stoechas* plants and micropropagated plantlets grown on MS medium supplemented with different BAP concentrations was evaluated using the disc diffusion method by measuring inhibition zones (mm). The activities against the phytopathogenic fungi *Alternaria* sp. *Acacia*, *Alternaria* sp. *Eucalyptus*, *Biscogniauxia mediterranea* and *Pestalotiopsis biciliata* (listed in Table 4) are summarized in Table 5. The results demonstrated considerable antifungal activity of the extracts from wild-growing plants (inhibition zone of 14.30 ± 0.44 mm) and those isolated from plants grown on media supplemented with 1 µM BAP (19.50 ± 0.05 mm) and 3 µM BAP (12.00 ± 0.40 mm) against *Alternaria* sp. *Acacia*. In contrast, antifungal activity comparable to the negative control (DMSO) was noted for all extracts against the *Alternaria* sp. *Eucalyptus* and *Biscogniauxia mediterranea* strains. The fungal strain *Pestalotiopsis biciliata* was the most resistant to all tested extracts (Table 5).

Table 4. Isolate codes, hosts, and GenBank accession numbers (ITS rDNA) of the studied phytopathogens.

Fungal Pathogen	Isolate Code	Host	GenBank Accession Number (ITS)
Alternaria sp.	Alt15	*Eucalyptus* sp.	—
Alternaria sp.	Alt40	*Acacia* sp.	—
Biscogniauxia mediterranea	BM.9	*Cork oak*	MT271046
Pestalotiopsis biciliata	TN.20	*Kermes oak*	MN841932

Table 5. Antifungal activity (zone of inhibition, mm) of methanolic extracts from wild-growing and in vitro propagated *L. stoechas* plants, against selected fungal strains (inhibition zone includes disc diameter of 6 mm; 25 µL of extract/disc).

Extract Source	BAP Concentration	Inhibition Zone (mm)			
		Alternaria* sp. *Acacia	***Alternaria* sp. *Eucalyptus***	***Biscogniauxia mediterranea***	***Pestalotiopsis biciliata***
Wild-growing	—	14.30 ± 0.44 b	3.30 ± 0.07 a	3.70 ± 0.20 a	0.00 ± 0.00 a
In vitro	0 µM	0.50 ± 0.05 a	4.00 ± 0.21 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	1 µM	19.50 ± 0.05 b	4.00 ± 0.30 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	2 µM	0.00 ± 0.00 a	4.70 ± 0.09 a	3.50 ± 0.15 a	0.00 ± 0.00 a
	3 µM	12.00 ± 0.40 ab	6.00 ± 0.25 a	4.50 ± 0.45 a	1.00 ± 0.10 a
DMSO Control	—	2.00 ± 0.00 a	3.00 ± 0.00 a	2.00 ± 0.00 a	0.00 ± 0.00 a

Values are means ± SE (n = 3). Different letters within a column indicate statistically significant differences between means at $p \leq 0.05$ according to Duncan's multiple range test.

4. Discussion

The high economic value of *L. stoechas* requires the optimization of in vitro seed germination techniques in order to ensure greater valorization of this species and the protection of its natural habitats. Therefore, the effects of sucrose concentration (1% and 2%) and temperature (20 °C and 25 °C) on in vitro seed germination were studied. The most rapid seed germination was achieved on a half-strength MS medium supplemented with 1% sucrose at 20 °C, where the first germinated seeds were observed on day 3 after sowing. The effect of temperature on *L. stoechas* seed germination has been demonstrated in previous studies [34,35]. Indeed, Maher et al. [34] found high germination percentages (over 70%) across a wide temperature range (10–25 °C). This can be explained by the fact that the temperature affects the germination of dormant seeds only within a specific range [36]. Regarding the effect of sucrose concentration in the culture medium, the lower concentration (1%) resulted in the highest germination percentage. Similarly, Kiani et al. [37] found that a half-strength MS medium with 1% sucrose was optimal for the germination of *Melissa officinalis* seeds. Different sugar concentrations can exert varying effects on germination processes via multiple pathways [38].

In vitro culture establishment and shoot multiplication were successfully achieved on a culture medium without PGRs. However, to further increase shoot proliferation, cytokinins were applied, as they are well-known inducers of cell division that promote shoot multiplication [39]. In this study, the morphological responses of nodal segment explants to various BAP concentrations were evaluated. The results showed a clear dose-dependent positive effect of BAP (1–3 μM) on the number of shoots (4.5–8.4 shoots per explant), leaves (24.5–37.2 per explant), and biomass production (18.9–24.0 mg DW per explant), compared to the control (3.5 shoots, 20.5 leaves and 13.8 mg DW per explant, respectively). The stimulatory effect of BAP on shoot multiplication has been widely documented in different lavender species. For example, in *L. pedunculata*, 1.11 μM BAP yielded a maximum of 4.07 shoots per explant [40], while in *L. coronopifolia*, 2.22 μM BAP produced 3.6 microshoots per explant [41]. Thus, our results indicate a substantially higher multiplication rate than those reported in these related species. Interestingly, in *L. stoechas*, Benyammi et al. [25] did not observe an increase in the number of shoots per explant when using BAP alone, but only when it was combined with GA_3, suggesting that GA_3 supplementation is required for optimal growth and proliferation.

Spontaneous rooting of the micropropagated shoots on the PGR-free medium occurred at a high frequency, a phenomenon also observed in other lavender species [40]. During acclimatization, survival rates exceeding 89% were recorded. Therefore, this study successfully established a reliable protocol for the in vitro propagation of *L. stoechas*.

In this study, we evaluated the qualitative and quantitative variations in phenolic compounds between wild-growing and in vitro cultured *L. stoechas* plants. The observed differences indicate that in vitro culture conditions and PGRs significantly affect the content of phenolic compounds, as the production of these metabolites is strongly influenced by ecological and edaphological factors [42]. Similar differences between wild-growing and in vitro cultured plants were also reported by Ghaderi et al. [43], who found a higher total phenolic content in in vitro cultured *Perovskia abrotanoides* plants compared to those from natural habitats. Another study found that micropropagated *Poliomintha glabrescens* plants had higher levels of phenolic compounds than wild plants [44]. However, Al Khateeb et al. [41] reported higher levels of phenolic compounds in wild plant extracts of *Lavandula coronopifolia*. Benyammi et al. [25] also recorded a higher total phenolic content in in vitro cultured *L. stoechas* plants compared to those from natural sources, although the authors did not indicate whether the observed difference was statistically significant. In addition, the aforementioned study did not examine the effects of different PGR treatments on total phenolic content.

HPLC analysis revealed that rosmarinic acid was the major phenolic acid present in all analyzed methanolic extracts. Other studies on methanolic extracts of *L. stoechas* have also identified rosmarinic acid as the major phenolic compound [6,45]. Similarly, Algieri et al. [46] reported the presence of rosmarinic acid in the phenolic profile of this species. In vitro culture approaches and the optimization of various experimental conditions have been widely employed for the production of this compound [17,47–49]. In the present study, the results showed that in vitro cultured plants produced this compound more efficiently than their wild-growing counterparts. These findings are consistent with those reported by Al Khateeb et al. [41], who found that in vitro grown microshoots and calli had the highest concentrations of rosmarinic acid in *L. coronopifolia*. The higher levels of rosmarinic acid in the vitro cultured plants, compared to those grown in natural habitats, could be attributed to the nutrient-rich composition of the culture medium [43]. While undifferentiated systems such as callus or cell suspension cultures can offer highly controlled and scalable production of rosmarinic acid and phenolics in many Lamiaceae species, our use of shoot cultures—leading to whole plantlets—was preferred because they maintain tissue differen-

tiation and organ-specific biosynthetic pathways more closely mimicking the intact plant. Moreover, shoot cultures provide superior genetic and biochemical stability with lower risk of somaclonal variation compared to dedifferentiated callus, while simultaneously supporting efficient micropropagation. These results suggest that in vitro propagation can promote the production of these phenolic compounds, highlighting the potential for further optimization in future research.

Remarkable differences in radical scavenging activity were detected between the methanolic extracts of wild-growing and in vitro cultured plants. Many previous studies have reported higher antioxidant capacity of extracts from in vitro cultures compared to wild-growing plants. This pattern was observed in *Lavandula viridis* [50], *Lavandula angustifolia* [51] and *Thymus lotocephalus* [52]. Similarly, Benyammi et al. [25] also showed higher antioxidant activity in the ethanolic extracts of in vitro cultured *L. stoechas* plants compared to wild-growing plants, although the statistical significance of these differences remains questionable. Furthermore, the effects of different PGR treatments on the antioxidant capacity of the extracts were not evaluated in their study. According to Mai et al. [53], the antioxidant activity of polyphenols is mainly due to their ability to act as hydrogen donors.

These findings support the important role of rosmarinic acid as a major contributor to the antioxidant capacity of *L. stoechas* extracts. Pearson's correlation analysis revealed strong positive correlations between rosmarinic acid content and DPPH ($r = 0.771$) as well as ABTS ($r = 0.844$) radical scavenging activities. Total phenolic content was also positively correlated with both assays. This is consistent with previous studies on Lamiaceae species and confirms that the enhanced antioxidant potential observed in in vitro cultured plants, particularly those treated with 3 µM BAP, is largely associated with the increased accumulation of rosmarinic acid.

When evaluating the concentration-dependent trends among the PGR treatments, an interesting physiological trade-off emerges between biomass accumulation and antioxidant capacity. While the maximum vegetative biomass was achieved at the highest concentration of 3 µM BAP, due to the established role of cytokinins in promoting active cell division and shoot proliferation, the superior antioxidant capacity was clearly exhibited in the lower BAP groups (1–2 µM). According to the growth-differentiation balance hypothesis, plant tissues constantly manage a metabolic trade-off where resource allocation to secondary pathways is negatively coupled with active growth objectives [54]. At 3 µM BAP, metabolic carbon resources are heavily partitioned toward cell division and structural growth. Conversely, under lower hormonal concentrations (especially 1 µM BAP), the reduced pressure for primary vegetative growth allows the cultured tissues to redirect excess carbon inputs into specialized metabolic pathways, such as phenolic and flavonoid biosynthesis, directly driving the higher antioxidant potential.

The use of plants rich in natural antifungal metabolites is considered an effective strategy to reduce the application of chemical pesticides and their associated environmental problems. Literature data show that the essential oil of *L. stoechas* generally exhibits low antifungal activity [55]. In contrast, Domingues et al. [56] reported strong antifungal activity of the essential oils of this species against fungi isolated from strawberry tree fruits. Neither ethanolic nor methanolic extracts of this plant showed any effects against *Candida albicans* [57,58]. To the best of the authors' knowledge, no previous studies have examined the effects of *L. stoechas* methanolic extracts on the phytopathogenic fungi tested in the present work. Although the extracts contained high levels of polar compounds such as phenols, tannins and flavonoids, the limited inhibitory efficacy against the tested fungi could be explained by the high stress tolerance and resistance of *Alternaria* and *Biscogniauxia* species [59,60]. The variations in the inhibition zones among the tested phytopathogenic strains can be attributed to differences in their cell wall architectures

and structural complexities. Fungi with highly chitinized or melanized cell walls often exhibit higher intrinsic tolerance to polar polyphenolic extracts, as these structural barriers limit the diffusion and cellular uptake of the active antifungal metabolites. Similarly, certain *Pestalotiopsis* isolates from extreme environments are known to produce bioactive metabolites [61].

5. Conclusions

In conclusion, the present study established a reliable protocol for the in vitro propagation of *L. stoechas* from seeds. In vitro culture conditions, combined with the application of BAP, significantly enhanced biomass production and total phenolic content, particularly the RA level. The recommended procedure initiates with seed germination on a half-strength MS medium supplemented with 1% sucrose at 20 °C, which yields the most rapid germination response. Subsequently, the cultivation of nodal segments on a full-strength MS medium with 3% sucrose and supplemented with 3 µM BAP represents the best overall treatment for shoot multiplication. This specific concentration was identified as the ideal compromise maximizing both biomass proliferation and bioactive metabolite accumulation, yielding the highest multiplication efficiency alongside the maximum production of RA.

Methanolic extracts from these optimized micropropagated plants exhibited high antioxidant activity, as assessed by the DPPH and ABTS assays (representing the second-best values among the tested treatments), which significantly exceeded the capacity of naturally grown plants. Furthermore, while the extracts generally showed limited or low overall efficacy against most of the tested phytopathogenic fungi, those derived from plants cultured on the optimized 3 µM BAP treatment displayed considerable and targeted antifungal activity specifically against *Alternaria* sp. *Acacia*. These findings demonstrate that in vitro propagation represents an effective strategy for improving the production of high-value bioactive metabolites while simultaneously promoting commercial exploitation, preventing the depletion of natural populations of Tunisian *Lavandula stoechas*, and providing a sustainable alternative to wild harvesting for future conservation efforts.

Future research should focus on the genetic stability of the micropropagated plants using molecular markers, the scale-up of biomass and RA production in temporary immersion bioreactors, and the use of elicitors to further enhance secondary metabolite accumulation. Exploring callus or cell suspension cultures could serve as a complementary strategy for large-scale, bioreactor-based optimization of targeted phenolic compounds. Field performance trials, essential oil profiling and comprehensive pharmacological evaluations will also be necessary to fully valorize this promising species.

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Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
BAP	6-benzylaminopurine
CA	Caffeic acid
CEs	Catechin equivalents
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
GAEs	Gallic acid equivalents
HPLC	High-performance liquid chromatography
MS	Murashige and Skoog medium
PGR	Plant growth regulator
QEs	Quercetin equivalents
RA	Rosmarinic acid
REs	Rutin equivalents
TEs	Trolox equivalents

References

1. Woronuk, G.; Demissie, Z.; Rheault, M.; Mahmoud, S. Biosynthesis and therapeutic properties of *Lavandula* essential oil constituents. *Planta Med.* **2011**, *77*, 7–15. [CrossRef] [PubMed]
2. Messaoud, C.; Chograni, H.; Boussaid, M. Chemical composition and antioxidant activities of essential oils and methanol extracts of three wild *Lavandula* L. species. *Nat. Prod. Res.* **2012**, *26*, 1976–1984. [CrossRef] [PubMed]
3. Sirohi, B.; Sagar, R. Effect of Hydroalcoholic Extract of *Dactylorhiza Hatagirea* Roots & *Lavandula Stoechas* Flower on Thiopental Sodium Induced Hypnosis in Mice. *J. Drug Deliv. Ther.* **2019**, *9*, 414–417. [CrossRef]
4. Bouyahya, A.; Et-Touys, A.; Abrini, J.; Talbaoui, A.; Fellah, H.; Bakri, Y.; Dakka, N. *Lavandula stoechas* essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities. *Biocatal. Agric. Biotechnol.* **2017**, *12*, 179–184. [CrossRef]
5. Karaca, N.; Demirci, B.; Demirci, F. Evaluation of *Lavandula stoechas* L. subsp. *stoechas* L., *Mentha spicata* L. subsp. *spicata* L. essential oils and their main components against sinusitis pathogens. *Z. Naturforsch.* **2018**, *73*, 353–360. [CrossRef] [PubMed]
6. Ceylan, Y.; Usta, K.; Usta, A.; Maltas, E.; Yildiz, S. Evaluation of antioxidant activity, phytochemicals and ESR analysis of *Lavandula stoechas*. *Acta Phys. Pol. A* **2015**, *128*, 483–487. [CrossRef]
7. Karabagias, I.K.; Karabagias, V.K.; Riganakos, K.A. Physico-Chemical Parameters, Phenolic Profile, In Vitro Antioxidant Activity and Volatile Compounds of Ladastacho (*Lavandula stoechas*) from the Region of Saidona. *Antioxidants* **2019**, *8*, 80. [CrossRef] [PubMed]
8. Gilani, A.H.; Aziz, N.; Khan, M.A.; Shaheena, F.; Jabeen, Q.; Siddiqui, B.S.; Herzig, J.W. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula stoechas* L. *J. Ethnopharmacol.* **2000**, *71*, 161–167. [CrossRef] [PubMed]
9. Behbahani, B.A.; Shahidi, F.; Mortazavi, A. Antimicrobial Effects of *Lavandula stoechas* L. and *Rosmarinus officinalis* L. Extracts on *Escherichia coli* and *Staphylococcus aureus*. *Sci. J. Microbiol.* **2013**, *2*, 15–22.
10. Canlı, K.; Yetgin, A.; Benek, A.; Bozyel, M.E.; Altuner, E.M. In Vitro Antimicrobial Activity Screening of Ethanol Extract of *Lavandula stoechas* and Investigation of Its Biochemical Composition. *Adv. Pharmacol. Sci.* **2019**, *2019*, 3201458. [CrossRef] [PubMed]

11. Kulabas, S.S.; Ipek, H.; Tufekci, A.R.; Arslan, S.; Demirtas, I.; Ekren, R.; Sezerman, U.; Tumer, T.B. Ameliorative potential of *Lavandula stoechas* in metabolic syndrome via multitarget interactions. *J. Ethnopharmacol.* **2018**, *223*, 88–98. [CrossRef] [PubMed]
12. Hmissi, S.; Chograni, H.; Yangui, I.; Riahi, L.; Boussaid, M.; Messaoud, C. Genetic diversity and population structure patterns in *Lavandula stoechas* L. wild germplasms using ISSR and EST-SSRs markers and implications for conservation. *Genet. Resour. Crop Evol.* **2023**, *71*, 2859–2876. [CrossRef]
13. Duda, M.M.; Imbrea, F.; Vârban, D.; Muntean, S. Research on values of 1000 seeds weight in some medicinal plants. *Res. J. Agric. Sci.* **2010**, *42*, 101–104.
14. Demirkaya, M.; Aydin, B.; Şekerci, A.D.; Gulsen, O. Effects of Osmotic Conditioning Treatments of Lavender (*Lavandula angustifolia*) Seeds on Mean Germination Time and Germination Rate. *Int. J. Sec. Metab.* **2017**, *4*, 418–422. [CrossRef]
15. Chavagnat, A. Lavender seed dormancy and germination. *Acta Hortic.* **1978**, *83*, 147–154. [CrossRef]
16. Saha, S.; Kader, A.; Sengupta, C.; Ghosh, P. In vitro propagation of *Ocimum gratissimum* L. (Lamiaceae) and its evaluation of genetic fidelity using RAPD marker. *Am. J. Plant Sci.* **2012**, *3*, 64–74. [CrossRef]
17. Abdel Rahman, R.A.I.; El-Wakil, H.E.D.; Abdelsalam, N.R.; Elsaadany, R.M.A. In-vitro production of rosmarinic acid from basil (*Ocimum basilicum* L.) and lemon balm (*Melissa officinalis* L.). *Middle East J. Appl. Sci.* **2015**, *5*, 47–51.
18. Karthik, D.; Viswanathan, P.; Anuradha, C.V. Administration of rosmarinic acid reduces cardiopathology and blood pressure through inhibition of p22phox NADPH oxidase in fructose-fed hypertensive rats. *J. Cardiovasc. Pharmacol.* **2011**, *58*, 514–521. [CrossRef] [PubMed]
19. Jin, X.; Liu, P.; Yang, F.; Zhang, Y.H.; Miao, D. Rosmarinic acid ameliorates depressive-like behaviors in a rat model of CUS and Up-regulates BDNF levels in the hippocampus and hippocampal-derived astrocytes. *Neurochem. Res.* **2013**, *38*, 1828–1837. [CrossRef] [PubMed]
20. Ozturk, H.; Ozturk, H.; Terzi, E.H.; Ozgen, U.; Duran, A.; Uygun, I. Protective effects of rosmarinic acid against renal ischaemia/reperfusion injury in rats. *J. Pak. Med. Assoc.* **2014**, *64*, 260–265. [PubMed]
21. Shan, Y.; Wang, D.D.; Xu, Y.X.; Wang, C.; Cao, L.; Liu, Y.S.; Zhu, C.Q. Aging as a Precipitating Factor in Chronic Restraint Stress-Induced Tau Aggregation Pathology, and the Protective Effects of Rosmarinic Acid. *J. Alzheimer's Dis.* **2016**, *49*, 829–844. [CrossRef] [PubMed]
22. Nadeem, M.; Imran, M.; Aslam Gondal, T.; Imran, A.; Shahbaz, M.; Amir, R.M.; Sajid, M.W.; Qaisrani, T.B.; Atif, M.; Hussain, G.; et al. Therapeutic potential of rosmarinic acid: A comprehensive review. *Appl. Sci.* **2019**, *9*, 3139. [CrossRef]
23. Calvo, M.C.; Segura, J. In vitro morphogenesis from explants of *Lavandula latifolia* and *Lavandula stoechas* seedlings. *Sci. Hortic.* **1988**, *36*, 131–137. [CrossRef]
24. Nobre, J. In vitro cloning and micropropagation of *Lavandula stoechas* from field-grown plants. *Plant Cell Tissue Organ Cult.* **1996**, *46*, 151–155. [CrossRef]
25. Benyammi, R.; Krimat, S.; Alili, M.; Bekhouche, M.; Touari, O.; Belalia, N.; Missoum, A.; Khelifi, L.; Morsli, A. In vitro propagation of algerian *Lavandula stoechas* and assessment of biochemical composition and their antioxidant activity. *Bull. Pharm. Sci.* **2023**, *46*, 899–911. [CrossRef]
26. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
27. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* **1965**, *16*, 144–158. [CrossRef]
28. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* **2006**, *97*, 654–660. [CrossRef]
29. Kumaran, A.; Karunakaran, R.J. In vitro antioxidant activities of methanol extracts of *Phyllanthus* species from India. *LWT Food Sci. Technol.* **2007**, *40*, 344–352. [CrossRef]
30. Oyedemi, S.O.; Bradley, G.; Afolayan, A.J. In vitro and in vivo antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. *Afr. J. Pharm. Pharmacol.* **2010**, *4*, 70–78.
31. Doshi, P.; Adsule, P.; Banerjee, K.; Oulkar, D. Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (*Vitis vinifera* L) by products. *J. Food Sci. Technol.* **2015**, *52*, 181–190. [CrossRef] [PubMed]
32. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
33. Baron, E.J.; Finegold, S.M. Methods for testing antimicrobial effectiveness. In *Diagnostic Microbiology*; Stephanie, M., Ed.; Mosby: Baltimore, MD, USA, 1990; pp. 171–194.
34. Maher, J.; Gerasopoulos, D.; Maloupa, E. Temperature and light effects on germination of *Lavandula stoechas* seeds. *Acta Hortic.* **2000**, *541*, 261–264. [CrossRef]
35. Ghaderi-Far, F.; Coşgun, Z.L.; Değirmenci, C.Ü.; Tüysüz, İ.; Ülgen, C.; Tavşanoğlu, Ç. Light and temperature requirements for germination in the Mediterranean shrub *Lavandula stoechas* (Lamiaceae). *Plant Biol.* **2021**, *23*, 992–999. [CrossRef] [PubMed]

36. Bewley, J.D.; Black, M. *Physiology and Biochemistry in Relation to Germination: Viability, Dormancy, and Environmental Control*; Springer: New York, NY, USA, 1982; Volume 2, p. 375. [CrossRef]
37. Kiani, M.; Younesikelaki, F.S.; Ebrahimzadeh, M.H.; Savitikadi, P.; Jogam, P.; Sadanandam, A. Studies on the Effect of Various Seed Surface Sterilization and Growing Media on the In-vitro Germination of Lemon Balm (*Melissa officinalis* L.). *Indian J. Sci. Technol.* **2017**, *10*, 3. [CrossRef]
38. Gibson, S.I. Control of plant development and gene expression by sugar signaling. *Curr. Opin. Plant Biol.* **2005**, *8*, 93–102. [CrossRef] [PubMed]
39. Yew, C.; Balakrishnan, B.; Balakrishnan, J.; Subramaniam, S. The effect of cytokinins on in vitro shoot length and multiplication of *Hymenocallis littoralis*. *J. Med. Plants Res.* **2010**, *4*, 2641–2646. [CrossRef]
40. Zuzarte, M.; Dinis, R.; Cavaleiro, A.M.; Salgueiro, L.R.; Canhoto, J.M. Trichomes, essential oils and in vitro propagation of *Lavandula pedunculata* (Lamiaceae). *Ind. Crops Prod.* **2010**, *32*, 580–587. [CrossRef]
41. Al Khateeb, W.; Kanaan, R.; El-Elimat, T.; Alu'datt, M.; Lahham, J.; El-Oqlah, A. In vitro Propagation, Genetic Stability, and Secondary Metabolite Analysis of Wild Lavender (*Lavandula coronopifolia* Poir.). *Hortic. Environ. Biotechnol.* **2017**, *58*, 393–405. [CrossRef]
42. Casagrande, M.; Zanela, J.; Wagner, A.; Busso, C.; Wouk, J.; Iurckevicz, G.; Montanher, P.F.; Yamashita, F.; Malfatti, C.R.M. Influence of time, temperature and solvent on the extraction of bioactive compounds of *Baccharis dracunculifolia*: In vitro antioxidant activity, antimicrobial potential, and phenolic compound quantification. *Ind. Crops Prod.* **2018**, *125*, 207–219. [CrossRef]
43. Ghaderi, S.; Nejad Ebrahimi, S.; Ahadi, H.; Eslambolchi Moghadam, S.; Mirjalili, M.H. In vitro propagation and phytochemical assessment of *Perovskia abrotanoides* Karel. (Lamiaceae)—A medicinally important source of phenolic compounds. *Biocatal. Agric. Biotechnol.* **2019**, *19*, 101113. [CrossRef]
44. Garcia-Pérez, E.; Uribe, J.; Lara, S. Luteolin content and antioxidant activity in micropropagated plants of *Poliomintha glabrescens* (Gray). *Plant Cell Tissue Organ Cult.* **2012**, *108*, 521–527. [CrossRef]
45. Celep, E.; Akyüz, S.; İnan, Y.; Yesilada, E. Assessment of potential bioavailability of major phenolic compounds in *Lavandula stoechas* L. ssp. *stoechas*. *Ind. Crops Prod.* **2018**, *118*, 111–117. [CrossRef]
46. Algieri, F.; Rodriguez-Nogales, A.; Vezza, T.; Garrido-Mesa, J.; Garrido-Mesa, N.; Utrilla, M.P.; González-Tejero, M.R.; Casares-Porcel, M.; Molero-Mesa, J.; Del Mar Contreras, M.; et al. Anti-inflammatory activity of hydroalcoholic extracts of *Lavandula dentata* L. and *Lavandula stoechas* L. *J. Ethnopharmacol.* **2016**, *190*, 142–158. [CrossRef] [PubMed]
47. Pavlov, A.I.; Georgiev, M.I.; Ilieva, M.P. Production of rosmarinic acid by *Lavandula vera* MM cell suspension in bioreactor: Effect of dissolved oxygen concentration and agitation. *World J. Microbiol. Biotechnol.* **2005**, *2*, 389–392. [CrossRef]
48. Yousefian, S.; Lohrasebi, T.; Farhadpour, M.; Haghbeen, K. Effect of methyl jasmonate on phenolic acids accumulation and the expression profile of their biosynthesis-related genes in *Mentha spicata* hairy root cultures. *Plant Cell Tissue Organ Cult.* **2020**, *142*, 285–297. [CrossRef]
49. Ejsmont, W.; Kiss, A.K.; Grzegorczyk-Karolak, I. Optimization of in vitro shoot culture parameters for enhanced biomass and rosmarinic acid production in *Salvia atropatana*. *Molecules* **2025**, *30*, 2654. [CrossRef] [PubMed]
50. Costa, P.; Gonçalves, S.; Valentão, P.; Andrade, P.B.; Romano, A. Accumulation of phenolic compounds in in vitro cultures and wild plants of *Lavandula viridis* L'Hér and their antioxidant and anticholinesterase potential. *Food Chem. Toxicol.* **2013**, *57*, 69–74. [CrossRef] [PubMed]
51. Andrys, D.; Kulpa, D.; Grzeszczuk, M.; Bihun, M.; Dobrowolska, A. Antioxidant and antimicrobial activities of *Lavandula angustifolia* Mill. field-grown and propagated in vitro. *Folia Hortic.* **2017**, *29*, 161–180. [CrossRef]
52. Costa, P.; Gonçalves, S.; Valentão, P.; Andrade, P.B.; Coelho, N.; Romano, A. *Thymus lotocephalus* wild plants and in vitro cultures produce different profiles of phenolic compounds with antioxidant activity. *Food Chem.* **2012**, *135*, 1253–1260. [CrossRef] [PubMed]
53. Mai, T.T.; Fumie, N.; Chuyen, N.V. Antioxidant activities and hypolipidemic effects of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) merr. and perry. *J. Food Biochem.* **2009**, *33*, 790–807. [CrossRef]
54. Korbozova, N.; Samarina, L.; Shadenova, E.; Dairbekova, D.; Yerbay, M.; Terletskaya, N. Temperature-Directed Reprogramming of Volatile and Semi-Volatile Metabolism in *Ginkgo biloba* Microclones Under Cold and Heat Stress. *Int. J. Mol. Sci.* **2026**, *27*, 3393. [CrossRef] [PubMed]
55. Zuzarte, M.; Gonçalves, M.J.; Cavaleiro, C.; Cruz, M.T.; Benzarti, A.; Marongiu, B.; Salgueiro, L. Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. *Ind. Crops Prod.* **2013**, *44*, 97–103. [CrossRef]
56. Domingues, J.; Delgado, F.; Gonçalves, J.C.; Pintado, C.S. Essential Oils of *Lavandula stoechas* subsp. *luisieri* as Antifungal Agent against Fungi from Strawberry Tree Fruit. *J. Pharm. Pharmacol.* **2021**, *9*, 98–106. [CrossRef]
57. Uzun, E.; Sariyar, G.; Adsersen, A.; Karakoc, B.; Ötük, G.; Oktayoglu, E.; Pirildar, S. Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. *J. Ethnopharmacol.* **2004**, *95*, 287–296. [CrossRef] [PubMed]
58. Bayrak, D.; Okmen, G.; Arslan, A. The Biological Activities of *Lavandula stoechas* L. against Food Pathogens. *Int. J. Sec. Metab.* **2017**, *4*, 270–279. [CrossRef]

59. Vannini, A.; Valentini, R. Influence of water relations on *Quercus cerris–Hypoxylon mediterraneum* interaction: A model of drought-induced susceptibility to a weakness parasite. *Tree Physiol.* **1994**, *14*, 129–139. [CrossRef] [PubMed]
60. Meng, J.-W.; He, D.-C.; Zhu, W.; Yang, L.-N.; Wu, E.-J.; Xie, J.-H.; Shang, L.-P.; Zhan, J. Human-Mediated Gene Flow Contributes to Metapopulation Genetic Structure of the Pathogenic Fungus *Alternaria alternata* from Potato. *Front. Plant Sci.* **2018**, *9*, 198. [CrossRef] [PubMed]
61. Tejesvi, M.V.; Nalini, M.S.; Mahesh, B.; Prakash, H.S.; Kini, K.R.; Shetty, H.S.; Subbiah, V. New hopes from endophytic fungal secondary metabolites. *Bol. Soc. Quím. Méx.* **2007**, *1*, 19–26.

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