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Antifungal Potential of *Salvia somalensis* Against *Botrytis cinerea* and *Colletotrichum coccodes*

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Abstract

Salvia somalensis Vatke is a source of specialized metabolites with potential biological and agrochemical applications. Previous studies on the dichloromethane plant surface extract demonstrated strong antifungal activity associated with abietane diterpenoids. Building on these findings, the present study aimed to characterize the methanolic extract of *S. somalensis* and evaluate its antifungal potential against major phytopathogenic fungi, with the broader goal of exploring the extract as a sustainable source of antifungal metabolites for crop protection. LC-MS analysis of the methanolic extract revealed a diverse phytochemical profile comprising phenolic acids, including caffeic acid, ferulic acid, and rosmarinic acid, as well as abietane-type diterpenoids such as carnosol, rosmanol, and carnosic acid. Quantitative analysis showed that carnosic acid was present only at low levels (0.09% *w*/*w* of fresh biomass). The extract exhibited antifungal activity against *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. More than 80% inhibition of mycelial growth was observed against *B. cinerea* and *C. coccodes* at 1000 µg/mL. In addition, as in vitro cultivation of this species has not previously been explored, micropropagated plants and callus culture were established. Phytochemical profiling revealed distinct chemical compositions in the in vitro biomass. Extracts obtained from micropropagated plants inhibited mycelial growth of the tested phytopathogens by 60–70% at 1000 µg/mL, except for *F. oxysporum*, which showed 35.29% inhibition at the same concentration. Collectively, these findings highlight *S. somalensis* as a source of bioactive metabolites with antifungal potential.

Keywords: *Salvia somalensis*; micropropagated plants; callus culture; LC-MS analysis; phytopathogenic fungi

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1. Introduction

The genus *Salvia* (Lamiaceae) comprises herbaceous and woody perennial species known for their aromatic properties and vast array of secondary metabolites with pharmaceutical potential [1,2]. Traditionally, various *Salvia* species have been used for their

antibacterial, antioxidant, antifungal, antitumor, and antidiabetic effects [3]. *Salvia somalensis*, native to Somalia and recently introduced as an ornamental species in Liguria, Italy, thrives in warm, well-drained soils and can be propagated by cuttings or clump division [4]. Previous phytochemical analyses of the dichloromethane surface extract of Ligurian *S. somalensis* revealed novel diterpenes, including 4*α*,9*α*-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one and high levels of carnosic acid, a bioactive abietane diterpenoid with potent antimicrobial properties against phytopathogens [5].

Phytopathogens represent a major constraint to agricultural productivity, significantly reducing crop yield and quality and causing substantial economic losses [6]. Although control strategies based on synthetic products remain widely adopted to manage plant diseases, their extensive use raises concerns related to the accumulation of residues in food products, adverse effects on non-target organisms, and broader environmental impacts [7]. Consequently, increasing attention has been directed toward plant-derived products as safer and more sustainable alternatives for the management of fungal and bacterial crop diseases [5,8]. Among these, diterpenoids from *Salvia* species have shown promising antifungal potential [9]. Notably, the dichloromethane surface extract of *S. somalensis* was previously found to be a rich source of carnosic acid (113.90 μg/mg of dried extract). Interestingly, complete inhibition of mycelial growth of *Colletotrichum coccodes*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* was observed after treatment with carnosic acid at 500 μg/mL [5].

Building on these promising findings, the present study focused on the characterization of the methanolic extract of *S. somalensis* and its antifungal activity against phytopathogenic fungi. The use of the whole extract may offer a more practical and accessible approach compared to the isolation of pure compounds such as carnosic acid, which requires additional extraction, purification, time, and cost. In addition, the complex mixture of phytochemicals present in the methanolic extract may act synergistically, contributing to the observed biological activity and supporting its potential use as a sustainable alternative for plant disease management [10]. To further explore differences in chemical composition and metabolite production, micropropagated plants and callus cultures of *S. somalensis* were also established. In vitro culture techniques provide a controlled environment for the stable, continuous production of plant biomass and bioactive metabolites year-round, thereby minimizing the effects of seasonal and environmental changes [11]. In addition, these approaches may be useful for producing valuable metabolites in situations where conventional agriculture may be limited by environmental stresses such as salinity, drought, climate change, and water scarcity [12]. Elicitors are employed to stimulate secondary metabolite biosynthesis through the activation of plant defence responses, significantly improving shoot proliferation and metabolite yield in *Salvia* explants [13]. Several *Salvia* species have been successfully cultured in vitro to produce valuable metabolites for pharmaceuticals, cosmetics, food, and crop protection [14,15]. However, no studies have documented the establishment of in vitro cultures or the secondary metabolite production profiles of *S. somalensis* in vitro biomass.

This study aims to (i) characterize the chemical profiles of methanolic extracts of *S. somalensis* and in vitro extracts, (ii) establish micropropagated plantlets and callus cultures of *S. somalensis* with and without elicitor treatments, (iii) quantify carnosic acid production in the extracts, and (iv) evaluate the antifungal potential of in vivo and in vitro extracts against phytopathogenic fungi, thereby contributing to the development of sustainable and environmentally friendly approaches to producing antifungal bioactive compounds.

2. Materials and Methods

2.1. Plant Material

The Centro Regionale di Sperimentazione ed Assistenza Agricola (CeRSAA), Albenga (SV), Italy, provided the fresh aerial material of *S. somalensis* plants. The plant material was identified by G.L.C. Bramley, with the deposit of a voucher specimen at the Kew Herbarium (K) being made under the INTERREG III ALCOTRA, Progetto No. 074 "Sviluppo a Scopi Commerciali delle Potenzialità del Genere *Salvia* L.".

The extraction was performed by immersing the fresh aerial plant (149.8 g) in methanol for 24 h, followed by filtration (extraction was repeated three times). The filtrate was subsequently evaporated to dryness under reduced pressure to ensure complete removal of the solvent (7.6 g).

2.2. Chemicals

Solvents for extraction and purification were purchased from VWR International Srl (Milano, Italy). Ultra-pure acetonitrile, water, methanol, and formic acid for LC-MS analysis were purchased from Romil Ltd., Pure Chemistry (Cambridge, UK). Deuterium oxide (D_2O, 99.90% D), CD_3OD (99.95% D), and 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (TSP) were purchased from Sigma-Aldrich Chemical Company (Milano, Italy). Carnosic acid (Product # 89171 CAS # 3650-09-7) was acquired from PhytoLab GmbH & Co. (KG, Vestenbergsgreuth, Germany). Commercial fungicides Ortiva® (azoxystrobin 23%) and Switch® (cyprodinil 37% + fludioxonil 25%) were acquired from Syngenta (Milano, Italy).

2.3. General Experimental Procedures

TLC, MPLC, and semi-preparative HPLC were performed as previously described [16]. Pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany) were used for TLC. $CHCl_3$-CH_3OH-HCOOH (10:0.5:0.1) was used as the mobile phase, and spots were detected by spraying 50% H_2SO_4, followed by heating. MPLC chromatography was performed on a Spot Liquid Chromatography system (Armen Instrument, Saint Ave, France) with Normal Phase Si60 Cartridges Supervarioflash and LiChroprep RP-18 (40–63 mm) (Merck, Darmstadt, Germany). Normal Phase Si60 Cartridges Supervarioflash (Merck, Darmstadt, Germany) were used for MPLC. A linear gradient was used, with *n*-hexane/$CHCl_3$/CH_3OH at concentrations varying from 100:0:0 to 0:0:100 serving as the elution mixture. Semi-preparative HPLC was carried out using a Waters W600 pump equipped with a Rheodyne Delta 600 injector, a 2414 refractive index detector, and a 2998 photodiode array detector (all Waters Corporation, Milford, MA, USA). A C18 SymmetryPrep column, with 7.8 × 300 mm ID and 7 µm particle size (Waters) was used for semi-preparative HPLC at room temperature, with a flow rate 1.03 mL/min and sample injection volume 100 µL. NMR data were recorded on a Bruker Avance III NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 500.13 MHz for ^1H and 125.77 MHz for ^{13}C nuclei. ^1H, ^{13}C, COSY, HSQC, HMBC, and NOESY NMR spectra were recorded in CD_3OD (ARMAR isotopes, 99.8 atom% D) or DMSO-d_6 (ARMAR isotopes, 99.9 atom% D) at 23 °C on a 5 mm BBO probe with a Z-gradient.

2.4. LC-MS Analysis

HPLC-PDA-CAD-ESIMS or APCIMS was conducted on an LC-MS 8030 chromatographic system (Shimadzu, Kyoto, Japan) consisting of a degasser, auto-sampler, quaternary pump (LC-20AD), a column oven (CTO-20AC), a PDA detector (SPD-M20A), a triple quadrupole MS, and a Dionex Corona Veo RS CAD detector (Thermo-Fisher Scientific, Germering, Germany). Ultimate300 UHPLC (Thermo Fisher Scientific, Milan, Italy) HPLC interfaced through an ESI source to a high-resolution mass analyser [(Orbitrap Q-Exactive

Classic Mass Spectrometer (Thermo Fisher Scientific Inc., Milan, Italy)] operating in both polarity, negative and positive ion mode. The MS data were acquired in full-mass and data-dependent-scan mode, then tandem MS experiments were performed to identify the specialized metabolites. A C18 column (Luna C18, Phenomenex, 150 × 2.0 mm, 3 µm) and a binary mobile phase composed of eluent A (ultrapure H_2O-HCOOH 0.1% v/v) and eluent B (ultrapure CH_3CN-HCOOH 0.1% v/v) were used. The separation conditions start first under isocratic conditions at 5% of B for 2 min; after this, a gradient to 50% occurred for 8 min, and then to 100% over 30 min. The flow rate was 0.20 mL/min, and the injection volume was 10.0 µL.

2.5. Callus Cultures and Micropropagation

Callus of *S. somalensis* was obtained using young leaves taken from the third and fourth nodes under the apical bud of plants grown in pots in the botanical garden of the University of Urbino Carlo Bo, Urbino (PU), Italy. The leaves were washed thoroughly under running tap water, then dried on absorbent paper. For surface sterilization, they were treated with 50% ethanol for 1 min, followed by 0.1% mercury chloride ($HgCl_2$) solution for 10 min [17]. After five rinses with sterile distilled water and drying with sterile filter paper, the leaves were transversely sectioned under aseptic conditions using a sterile scalpel. To induce the callus formation, the Murashige and Skoog (MS) medium [18] was supplemented with 1.13 µM of 2,4-dichlorophenoxyacetic acid (2,4-D) and 30 g/L sucrose. The pH of the medium was adjusted to 5.8 before the addition of agar (0.8%), and the medium was then autoclaved at 120 °C and 104 kPa for 20 min and dispensed into 90 mm Petri dishes (30 mL per Petri dish). Seven sterile leaf explants were placed in each Petri dish, and the cultures were maintained in a climatic cell at 25 °C for a photoperiod of 16 h obtained with fluorescent tubes at a light intensity of 60 µmol $m^{-2}s^{-1}$. After thirty days, the small portions of callus that developed at the cutting surface of the explants were excised and transferred to fresh culture medium. Subculturing was carried out using MS medium prepared in a similar way as that described above. Subsequent subcultures were performed at 30-day intervals to promote callus proliferation. A friable white and green callus was successfully established. Following the third subculture, the callus biomass (277.5 g) was stored at −20 °C and subjected to lyophilization (9.25 g). The freeze-dried material was then extracted with methanol (1.14 g). In addition to standard callus culture, *S. somalensis* calluses were also elicited with salicylic acid. For elicitation, callus induction was carried out on MS medium supplemented with 50.0 µM salicylic acid. The elicited callus (254.9 g) was then lyophilized (8.53 g) and subjected to methanolic extraction (1.0 g).

For the in vitro micropropagation of *S. somalensis*, nodal segments of around 5 mm length were excised from the third and fourth nodes of plants. Surface sterilization was performed as described above. The sterilized explants were then cultured on MS medium without growth regulators and medium containing two growth regulators, 0.5 mg/L of 6-benzylaminopurine (BA) and 0.1 mg/L of α-naphthalene acetic acid (NAA) [19]. Both media contained 3% sucrose and 0.8% agar. A total of 15 nodal explants were placed in glass jars containing MS medium (10 cm diameter × 11 cm height). The pots were placed in a climatic cell at 22 ± 2 °C, 16/8 photoperiod, 60 µM $m^{-2}s^{-1}$ light intensity. After 5 weeks, the shoots obtained from some explants were excised and subcultured in a new culture medium with the same conditions to maintain the micropropagation. Following a ten-week growth period, the micropropagated biomass (410.0 g) was harvested from the culture vessels, frozen, and lyophilized before extraction.

2.6. Chemical Investigation of the Extract of Micropropagated Plants

The freeze-dried micropropagated biomass (23.4 g) was exhaustively extracted with methanol, following a previously reported method [20]. After filtration, the extraction solvent was removed under reduced pressure. The extract (8.5 g, 2.07% *w*/*w* of fresh plant) was chromatographed in aliquots of 1.0 g by Si gel MPLC eluted with *n*-hexane/$CHCl_3$/CH_3OH at concentrations varying from 100:0:0 to 0:0. The eluate fractions (20 mL each) were combined according to their TLC patterns to yield 28 fractions (I_1–I_{28}) (group I). HPLC purification (linear gradient from 60% CH_3CN in H_2O to 100% CH_3CN occurred over 30 min, followed by elution with 100% CH_3CN to 50 min) of fraction I_9 (26.0 mg), which afforded **1** (2.4 mg), **2** (1.5 mg), and **3** (2 mg). Fraction I_{17} (59.8 mg) afforded **1** (2 mg) and **2** (2.7 mg). Fraction I_{21} (54.2 mg) afforded **3** (3.4 mg). The fractionation of fraction I_{25} (495.1 mg) afforded 10 fractions ($II_{1–10}$) (group II). Fraction II_4 (150.0 mg) was purified by semi-preparative HPLC (linear gradient from 60% CH_3OH in H_2O to 100% CH_3OH over 61 min, followed by elution with 100% CH_3OH until 90 min). HPLC purification of fraction II_4 afforded **1** (4 mg), **2** (5.2 mg), **3** (1 mg), **4** (1.4 mg), **5** (3.4 mg), and **6** (7.6 mg). The fractionation of fraction I_{26} (502.2 mg) afforded 10 fractions ($III_{1–10}$) (group III). HPLC purification (linear gradient from 60% CH_3CN in H_2O to 100% CH_3CN for 30 min, followed by elution with 100% CH_3CN until 50 min) of fraction III_5 (173.4 mg) afforded **1** (18.5 mg) and **2** (13.8 mg). The fractionation of I_{27} (900.1 mg) afforded 13 fractions ($IV_{1–13}$) (group IV). Fraction IV_5 (220.0 mg) was purified by semi-preparative HPLC (linear gradient from 60% CH_3OH in H_2O to 100% CH_3OH for 61 min, followed by elution with 100% CH_3OH for 90 min) affording **1** (0.8 mg), **2** (1 mg), **3** (1 mg), **4** (0.9 mg), **5** (1.1 mg), **6** (2.2 mg), and **7** (1.0 mg).

2.7. HPLC Quantitative Analysis

The quantification of carnosic acid (**1**) was carried out through analytical HPLC (Thermo Scientific™, Waltham, MA, USA) equipped with a Symmetry C_{18} column (1 mm × 150 mm, 3.5 µm particle size, Waters S.p.A., Sesto San Giovanni, Italy) at room temperature, following the previously validated method described by Paloukopoulou et al. [21] which was applied without modification. Gradient chromatography was carried out using a binary solvent system of eluent A (H_2O, water) and eluent B (CH_3CN, acetonitrile). Gradient shape was initiated with an isocratic hold at 50% B from 0 to 10 min, then raised linearly to 58% B (10–20 min). This mixture was maintained for another 10 min (20–30 min). Then, the gradient was incremented to 100% B in the next 10 min (30–40 min), and this final composition was maintained at a fixed level for a duration of 20 min, followed by re-equilibration of the column with 50% B. The flow rate was 0.400 mL/min and the injection volume was 20 µL. UV detection, monitored at 280 nm. The calibration curve for carnosic acid was obtained at a concentration range from 0.1 mg/mL to 1.0 mg/mL. The linearity of the instrumental response in the analyzed concentration range was confirmed, as inferred by the following fitting curve parameters: $y = 111.27x + 7.2932$; $R^2 = 0.9956$. The limit of detection (LOD) and limit of quantification (LOQ) were determined by the serial dilution of carnosic acid (**1**) until the signal-to-noise (S/N) ratio was 3 for LOD and 10 for LOQ. The obtained values of LOD and LOQ were 0.27 mg/mL and 0.08 mg/mL, respectively.

2.8. Antifungal Activity

Phytopathogenic strains of *Colletotrichum coccodes*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Botrytis cinerea* were isolated from symptomatic plants of *Solanum lycopersicum*, *Cyclamen persicum*, *Lactuca sativa*, *Ocimum basilicum* and *Fragraria* × *ananassa*, respectively, at CeRSAA (Albenga, SV), Italy. The isolation and identification of strains were performed as described in our previous work on *S. somalensis* [5]. The

antifungal properties of *S. somalensis* were tested using the food-poisoning method [22,23]. Methanolic extracts (two concentrations, 500 and 1000 µg/mL, respectively) were prepared from in vivo plants, micropropagated plants, callus cultures, and elicitor-treated callus cultures. The efficacy of the extracts was tested against the mycelial growth of selected phytopathogenic fungi. Potato dextrose agar (PDA) was prepared by autoclaving 39 g of medium dissolved in 1 L of distilled water at 121 °C for 15 min. Once cooled to approximately 50 °C, the extract dissolved in 0.75% *v*/*v* dimethyl sulfoxide (DMSO) was incorporated into the medium at concentrations of 500 and 1000 µg/mL. Streptomycin sulfate (5 µg/mL) was added to control bacterial proliferation, and then the medium was poured into 90 mm Petri dishes. Commercial fungicides: Ortiva® (azoxystrobin 23%) at 250 µg/mL and Switch® (cyprodinil 37% + fludioxonil 25%) at 375 µg/mL and 250 µg/mL were included as reference treatments. An agar plug (2 × 2 mm) inoculated with actively growing fungal cultures was placed at the centre of each plate and incubated in complete darkness at 25 °C. All treatments were conducted in triplicate in parallel with controls containing media with 0.75% *v*/*v* DMSO and untreated PDA. Fungal growth was measured by the diameter of the colonies, and the inhibition was calculated using the following formula: percentage inhibition (%) = [(NT − T)/NT] × 100, where NT is the average colony diameter in the untreated samples and T is the average diameter in the treated ones. All experiments were done three times independently, and the results were consistent across all trials. Dose–response analysis was performed using five concentrations (100, 250, 500, 750, and 1000 µg/mL), each tested in triplicate. The inhibitory effect was expressed as percentage inhibition, calculated using Abbott's formula. The dose–response curves were fitted using a four-parameter logistic (4PL) regression model according to the equation $y = \min + (\max - \min) / [1 + (x/IC_{50})^{\text{Hill slope}}]$ for *C. coccodes*, *F. oxysporum*, *S. sclerotiorum*, *R. solani* and an exponential saturation model according to the equation $y = \min + (\max - \min)(1 - e^{-kx})$ for *B. cinerea*. Data analysis and curve-fitting were performed using MATLAB R2026a (The MathWorks, Inc., Natick, MA, USA).

2.9. Statistical Analysis

Statistical analyses were performed using MINITAB® software (Version 19.2020.1, 64-bit; LLC, State College, PA, USA). The data obtained from the food-poisoning assay were analyzed using one-way ANOVA to determine whether there were significant differences among the different treatment groups. The ANOVA results were further subjected to Tukey's honest significant difference (HSD) test at a confidence level of $p \leq 0.05$. Three independent replicates were used for each treatment group.

3. Results

3.1. Establishment of Callus Cultures and Micropropagation

Friable white-green callus (Figure S1, Supplementary Materials) was obtained in the culture medium supplemented with an auxin, 2,4-D (1.13 µM), a growth regulator used for callus induction [24,25], both in the absence and presence of the elicitor (salicylic acid 50.0 µM). In both conditions, the callus showed no signs of differentiation or organogenesis. However, through regular subculturing, the callus remained stable over time without exhibiting ageing symptoms such as browning or necrosis.

For micropropagation, when nodal explants of *S. somalensis* were placed in a medium without growth regulators, they failed to produce shoots and died within one week. On the other hand, explants cultured on a medium supplemented with 0.5 mg/L BA and 0.1 NAA produced an average of 7.43 ± 0.84 shoots per explant. This medium was prepared according to the protocol developed by Petrova et al. [19] for the in vitro propagation of *S. officinalis*. BA is commonly utilized along with low concentrations of NAA to enhance morphogenesis.

The combination of BA and NAA for our study was selected based on previously developed protocols for other *Salvia* species' micropropagation [26–29]. Higher concentrations of BA may result in hyperhydricity, while lower concentrations have been shown to increase shoot proliferation from nodal explants without affecting plant quality [30–32]. In our study, BA at a concentration of 0.5 mg/L successfully boosted shoot development without any visible signs of hyperhydricity (Figure S1 Supplementary Materials).

3.2. LC-MS Profiling of Methanolic Extracts from Different Sources of Salvia somalensis: In Vivo Biomass, Micropropagated Plant Biomass, Callus Cultures, and Elicitor-Treated Callus Cultures

To comprehensively characterize the chemical profile of *S. somalensis*, an LC-MS analysis was carried out on methanolic extracts from in vivo plant material, micropropagated plant, and callus cultures (with and without elicitor), in both positive and negative ionization modes (Figure S2, Supplementary Materials). LC-MS analysis revealed that the methanolic extract was dominated by terpenoids and flavonoids. The metabolites were identified using standards where available. The results confirmed the presence of several previously reported compounds from *S. somalensis* leaf surface exudate (Table 1) [5], along with additional metabolites distinctive of in vitro cultures. The in vivo plant extract was characterized by a rich composition of phenolic acids and abietane-type diterpenoids. Major ions corresponding to carnosic acid (**1**) (m/z 331 $[M-H]^-$), carnosol (**2**) (m/z 329 $[M-H]^-$), rosmanol (**4**) (m/z 345 $[M-H]^-$), and rosmarinic acid (m/z 359 $[M-H]^-$) were detected, together with caffeic acid, ferulic acid, and ermanin. In the micropropagated plant and callus extracts, LC-MS analysis revealed the presence of an abundant profile of diterpenoids and triterpenoids. Sesquiterpenoid ions detected in the negative ion mode at m/z 221.18 were attributed to (−)-7-*epi*-isojunenol, (+)-juneol, and (+)-*ent*-epicubenol, while ions at m/z 239 in the positive mode corresponded to 1β,6β-dihydroxy-4(14)-eudesmene. Triterpenoids such as betulin, uvaol (m/z 443), and betulinic acid (m/z 457) were also identified. The flavonoid 5-hydroxy-7,4′-dimethoxyflavone, previously isolated from *S. somalensis* leaf exudate [5], was detected at m/z 299. Diterpenoid compounds, including 7,13-*E*-labdadien-15-ol, pisiferol, rosmadial, 12-*O*-methylacetylcarnosate, 14-hydroxy-7-*O*-methylrosmanol, 12-methylcarnosol, galdosol, 20-deoxocarnosol, ferruginol, brussonol, and demethylsalvicanol, were identified mainly in micropropagated. Additionally, 4α,9α-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one, previously isolated for the first time in *S. somalensis* dichloromethane plant surface extract [5], was also detected in micropropagated plants and callus extracts. The callus culture without an elicitor showed ions at m/z 317 corresponding to 4β,6β-dihydroxy-1α,5α(H)-guai-9-ene and teucladiol, which were absent in micropropagated plants. Upon elicitor treatment, additional metabolites appeared, such as 11-hydroxy-12-methoxyabieta-8,11,13-triene and β-chaenocephalol, along with the presence of teucladiol, indicating the elicitor-induced activation of diterpenoid biosynthesis. Overall, the LC-MS results highlight a distinct metabolite pattern between the in vivo and in vitro systems, with the in vivo extract enriched in phenolic acids and abietane diterpenoids, while the micropropagated plant extract and elicited callus showed a broader diterpenoid–triterpenoid profile, suggesting metabolic diversification under in vitro and elicited conditions.

Table 1. LC-MS analysis of *Salvia somalensis* methanolic extracts of in vivo plant, micropropagated plant, callus and elicited callus.

Code	Compound Name	Chemical Formula	$[M+H]^+$	$[M-H]^-$	MS2	MSI [1]	Rt	In Vivo Plant [2]	Micropropagated Plant [2]	Callus [2]	Elicited Callus [2]
1	caffeic acid	$C_9H_8O_4$		179.0338	135	1	10.44	X	-	-	-
2	carnosic acid	$C_{20}H_{28}O_4$	333.2060	331.1903	289; 287	1	28.98	X	X	X	-
3	carnosol	$C_{20}H_{26}O_4$		329.1747	285	1	22.29	X	X	X	X
4	ermanin	$C_{17}H_{14}O_6$		313.0706	269	1	14.47	X	-	-	-
5	ferulic acid	$C_{10}H_{10}O_4$		193.0500	178; 161; 134	1	13.01	X	-	-	-
6	hydroxyjasmonic-*O*-hexoside	$C_{18}H_{28}O_9$		387.1667	225; 207; 163	1	10.13	X	-	-	-
7	isorhamnetin	$C_{16}H_{12}O_7$		315.0852	300	1	16.59	X	-	-	-
8	7-methylrosmanol	$C_{21}H_{28}O_5$		359.1863	329; 301; 283; 257	1	21.11	X	-	-	-
9	rosmarinic acid	$C_{18}H_{16}O_8$		359.0775	197; 179; 161; 135	1	12.32	X	-	-	-
10	rosmarinic acid isomer	$C_{18}H_{16}O_8$		359.0775	197; 179; 161; 135	1	11.73	X	-	-	-
11	12-methylcarnosic acid	$C_{21}H_{30}O_4$	347.2216		301; 286	1	41.66	-	X	X	X
12	rosmanol	$C_{20}H_{26}O_5$	347.1853	345.1708	301; 283; 257	1	16.87	X	X	X	X
13	rosmanol isomer	$C_{20}H_{26}O_5$		345.1708	301; 283; 257	1	17.51	X	-	-	-
14	oleanolic acid	$C_{30}H_{48}O_3$		455.3519	437	1	45.48	-	X	X	X

Table 1. *Cont.*

Code	Compound Name	Chemical Formula	[M+H]$^+$	[M−H]$^-$	MS2	MSI [1]	Rt	In Vivo Plant [2]	Micropropagated Plant [2]	Callus [2]	Elicited Callus [2]
15	ursolic acid	$C_{30}H_{48}O_3$	457.3676	455.3519	437	1	46.43	-	X	X	X
16	(−)-7-*epi*-isojunenol	$C_{15}H_{26}O$		221.1899	203	1	38.94	-	X	-	X
17	(+)-junenol	$C_{15}H_{26}O$		221.1899	203	1	43.01	-	X	-	X
18	(+)-*ent*-epicubenol	$C_{15}H_{26}O$		221.1899	203	1	44.91	-	X	-	X
19	1β,6β-dihydroxy-4(14)-eudesmene	$C_{15}H_{25}O_2$	239.2005		221; 203; 160	1	36.30	-	X	X	-
20	betulin	$C_{30}H_{50}O_2$	443.3883		425	1	48.10	-	X	X	-
21	uvaol	$C_{30}H_{50}O_2$	443.3883		425	1	48.58	-	X	X	-
22	betulinic acid	$C_{30}H_{50}O_3$		457.3676	439	1	47.38	-	X	-	-
23	5-hydroxy-7,4′-dimethoxyflavone	$C_{17}H_{14}O_5$	299.0914		281; 266; 251	1	37.63	-	X	-	X
24	7,13-*E*-labdadien-15-ol	$C_{20}H_{34}O$	291.2682		273; 255	1	47.82	-	X	-	-
25	pisiferol	$C_{20}H_{30}O_2$	303.2318		285	1	44.03	-	X	X	X
26	rosmadial	$C_{20}H_{24}O_5$	345.1696	343.1618	299; 197; 179; 145	1	13.09	X	X	X	X
27	12-*O*-methylacetylcarnosate	$C_{21}H_{28}O_3$	329.2111	327.1950	312; 269	1	44.17	-	X	-	-
28	14-hydroxy-7-*O*-methylrosmanol	$C_{21}H_{28}O_6$	377.1958		359; 344	1	38.82	-	X	-	X
29	12-methylcarnosol	$C_{21}H_{28}O_4$		343.1903	328	1	40.08	-	X	-	-
30	galdosol	$C_{20}H_{24}O_5$	345.1696		299; 243	1	36.11	-	X	X	X
31	20-deoxocarnosol	$C_{20}H_{28}O_3$	317.2111	315.1954	287; 269	1	33.68	-	X	X	X

Table 1. *Cont.*

Code	Compound Name	Chemical Formula	[M+H]$^+$	[M−H]$^-$	MS2	MSI [1]	Rt	In Vivo Plant [2]	Micropropagated Plant [2]	Callus [2]	Elicited Callus [2]
32	ferruginol	$C_{20}H_{30}O$	287.2369	285.2212	244; 269	1	39.29	-	X	X	X
33	brussonol	$C_{20}H_{28}O_3$	317.2111		299	1	38.65	-	X	-	-
34	demethylsalvicanol	$C_{20}H_{30}O_3$	319.2267		301	1	43.36	-	X	X	X
35	4*α*,9*α*-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one	$C_{20}H_{28}O_3$	317.2111		299	1	39.43	-	X	X	X
36	4*β*,6*β*-dihydroxy-1*α*,5*α*(H)-guai-9-ene	$C_{15}H_{26}O_2$	239.2005		221; 203	1	38.21	-	-	X	-
37	teucladiol	$C_{15}H_{26}O_2$	239.2005		221; 203	1	38.82	-	-		X
38	11-hydroxy-12-methoxyabieta-8,11,13-triene	$C_{21}H_{32}O_2$	317.2475		299	1	43.16	-	-	-	X
39	*β*-chaenocephalol	$C_{15}H_{26}O_2$	239.2005		221; 203	1	23.35	-	-	-	X
40	Triterpene	$C_{30}H_{48}O_4$		471.3484	453	3	24.83	X	-	-	-
41	Triterpene	$C_{30}H_{48}O_4$		471.3484	453	3	25.09	X	-	-	-
42	Triterpene	$C_{30}H_{48}O_5$		487.3432	469; 443	3	19.26	X	-	-	-

[1] MSI level of identification according to Sumner et al. [33]. [2] X indicates presence; - indicates absence.

3.3. Phytochemical Analysis of Micropropagated Plant Extract

The chemical investigation of the methanolic extract of micropropagated plants afforded several known compounds, identified by NMR (1D and 2D) and ESI-HRMS. The chromatographic separation afforded several diterpenoid compounds: carnosic acid (**1**) [34], carnosol (**2**) [34], 12-methylcarnosic acid (**3**) [34,35], rosmanol (**4**) [36,37], two triterpenoids, oleanolic (**5**) and ursolic acids (**6**) [38], and 7-methyl rosmanol (**7**) [39] (Table S1, Supplementary Materials).

3.4. Determination of the Content of Carnosic Acid

The content of carnosic acid in *S. somalensis* samples was quantified using HPLC-UV, based on calibration with an authentic standard. Carnosic acid was detected in both in vivo and micropropagated plant extracts, with concentrations corresponding to 0.09% and 0.07% (*w*/*w*) of fresh biomass, respectively. No detectable amount of carnosic acid was found in the callus samples, indicating quantities below the detection threshold.

3.5. Antifungal Screening

Antifungal activity of methanolic extracts obtained from *S. somalensis* was evaluated against five phytopathogenic fungi: *Botrytis cinerea*, *Colletotrichum coccodes*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. Four different methanolic types of extracts were tested—in vivo plant, micropropagated plants, callus, and elicitor-treated callus extracts—at concentrations of 500 and 1000 µg/mL. Two commercial fungicides, azoxystrobin (250 µg/mL) and cyprodinil + fludioxonil (375 + 250 µg/mL), were used as reference standards, and 0.75% *v*/*v* DMSO-treated and untreated PDA were used as negative controls. The results are shown in Table 2. The in vivo plant extract resulted in the highest antifungal activity against all tested fungi (Figures S3–S6, Supplementary Materials). It inhibited mycelial growth of *C. coccodes* at 1000 µg/mL by 86.27%, slightly lower than azoxystrobin (87.3%). The extract also showed strong inhibition against *B. cinerea* (84.3%; Figure 1) and inhibited *F. oxysporum* mycelial growth by 71.6% at the highest tested concentration. Cyprodinil + fludioxonil controlled 85.3% of *F. oxysporum* mycelial growth, and this inhibitory rate was significantly higher than that of azoxystrobin. The inhibition against *S. sclerotiorum* was 74.5% (1000 µg/mL) and 66.7% (500 µg/mL), showing a significantly higher antifungal activity than the chemical control azoxystrobin (57.8%). Mycelial growth suppression of 77.5% against *R. solani* was observed at 1000 µg/mL, which was statistically higher than the azoxystrobin effect. The micropropagated plant extract also demonstrated notable antifungal activity at 1000 µg/mL, with 63.7% control of *B. cinerea* mycelial growth and 68.6% inhibition against *R. solani*. The extract also controlled 62.8% and 61.8% mycelial growth of *C. coccodes* and *S. sclerotiorum*, respectively, at 1000 µg/mL. However, the efficacy was statistically lower than that of the commercial fungicides. The lowest activity was reported against *F. oxysporum* (35.3%). Methanolic extracts of callus culture and elicitor-treated callus culture showed low-level to no antifungal activity, with inhibition percentages ranging from 0% to 24.5%.

The estimated parameters of dose–response curves for all treatments are summarized in Tables S2 and S3 (Supplementary Information). Overall, the models showed good to excellent fits (R^2 = 0.955–0.998). Based on the four-parameter logistic model, *C. coccodes* and *R. solani* were the most sensitive species, with IC_{50} values of 304.91 µg/mL (in vivo plant) and 318.54 µg/mL (micropropagated plant), and 304.44 µg/mL (in vivo plant) and 307.80 µg/mL (micropropagated plant), respectively. *F. oxysporum* was the least sensitive, showing the highest IC_{50} value (613.60 µg/mL). For *F. oxysporum* treated with extracts from micropropagated plants, the IC_{50} could not be estimated because 50% inhibition was not achieved at the highest concentration tested. According to the exponential saturation model,

B. cinerea was more sensitive to extracts from micropropagated plants (IC_{50} = 52.49 µg/mL) than to extracts from in vivo plants (IC_{50} = 137.89 µg/mL).

Table 2. Antifungal activity of methanolic extracts of in vivo plant, micropropagated plant, callus cultures, and elicited callus cultures of *Salvia somalensis* against selected phytopathogenic fungi [a].

Treatment	Concentration	*Botrytis cinerea* (%) ± SD	*Colletotrichum coccodes* (%) ± SD	*Fusarium oxysporum* (%) ± SD	*Rhizoctonia solani* (%) ± SD	*Sclerotinia sclerotiorum* (%) ± SD
in vivo plant	500 µg/mL	77.45 ± 0.15 [d]	81.37 ± 0.06 [d]	71.57 ± 0.23 [b]	76.47 ± 0.10 [c]	66.67 ± 0.15 [c]
in vivo plant	1000 µg/mL	84.31 ± 0.06 [c]	86.27 ± 0.06 [c]	71.57 ± 0.25 [b]	77.45 ± 0.06 [b]	74.51 ± 0.06 [b]
micropropagated plant	500 µg/mL	62.75 ± 0.12 [e]	52.94 ± 0.26 [f]	19.61 ± 0.29 [g]	64.71 ± 0.10 [e]	52.94 ± 0.10 [f]
micropropagated plant	1000 µg/mL	63.73 ± 0.15 [f]	62.75 ± 0.21 [e]	35.29 ± 0.17 [d]	68.63 ± 0.15 [d]	61.76 ± 0.10 [d]
callus cultures	500 µg/mL	0.00 ± 0.00 [g]	0.00 ± 0.00 [i]	17.67 ± 0.49 [h]	19.61 ± 0.06 [i]	0.00 ± 0.00 [g]
callus cultures	1000 µg/mL	0.00 ± 0.00 [g]	0.00 ± 0.00 [i]	19.61 ± 0.15 [g]	24.51 ± 0.12 [g]	0.00 ± 0.00 [g]
elicited callus cultures	500 µg/mL	0.00 ± 0.00 [g]	22.55 ± 0.12 [h]	22.55 ± 0.25 [f]	18.63 ± 0.06 [j]	0.00 ± 0.00 [g]
elicited callus cultures	1000 µg/mL	0.00 ± 0.00 [g]	24.51 ± 0.29 [g]	24.51 ± 0.23 [e]	20.59 ± 0.10 [h]	0.00 ± 0.00 [g]
DMSO	7.5 µL/mL	0.00 ± 0.00 [g]	0.00 ± 0.00 [i]	0.00 ± 0.00 [i]	0.00 ± 0.00 [k]	0.00 ± 0.00 [g]
cyprodinil + fludioxonil	375 + 250 µg/mL	100.00 ± 0.00 [g]	100.00 ± 0.00 [a]	85.29 ± 0.26 [a]	100.00 ± 0.00 [a]	100.00 ± 0.0 [a]
azoxystrobin	250 µg/mL	88.24 ± 0.00 [b]	87.25 ± 0.06 [b]	47.06 ± 0.30 [c]	51.96 ± 0.12 [f]	57.84 ± 0.06 [e]
control	-	0.00 ± 0.00 [g]	0.00 ± 0.00 [i]	0.00 ± 0.00 [d]	0.00 ± 0.00 [k]	0.00 ± 0.00 [g]

[a] Values are expressed as the mean ± standard deviation of mycelial growth inhibition (%). Different letters represent significant differences at $p \leq 0.05$ by Tukey's test.

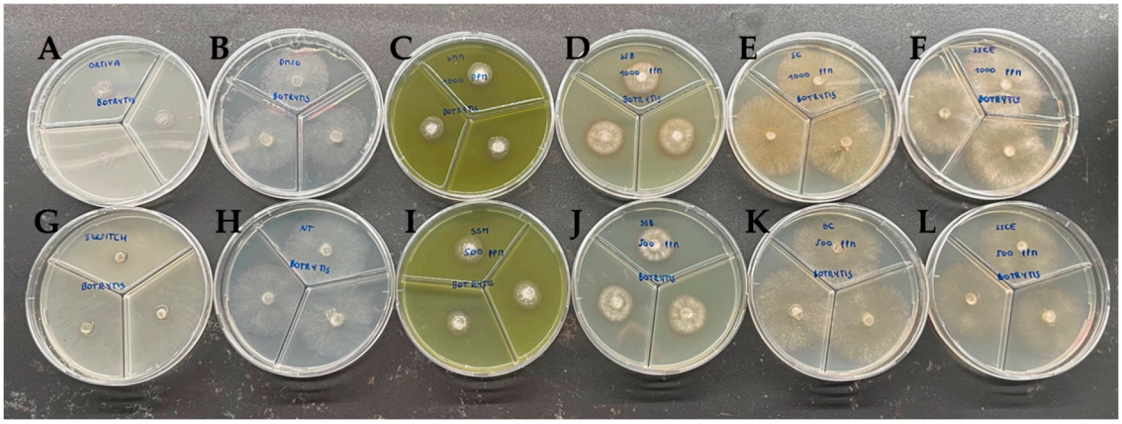

Figure 1. Representative inhibition of mycelial growth of *Botrytis cinerea*. (**A**) Azoxystrobin (250 µg/mL); (**B**) DMSO, (**C**) in vivo plant 1000 µg/mL; (**D**) micropropagated plant 1000 µg/mL; (**E**) callus 1000 µg/mL; (**F**) elicitor-treated callus 1000 µg/mL; (**G**) Cyprodinil + Fludioxonil 375 + 250 µg/mL; (**H**) Control; (**I**) in vivo plant 500 µg/mL; (**J**) micropropagated plant 500 µg/mL; (**K**) callus 500 µg/mL; (**L**) elicitor-treated callus 500 µg/mL.

4. Discussion

Research on plant-derived bioactive compounds offers promising, low-toxicity solutions for managing plant diseases, which aligns well with the objectives of sustainable agriculture [40]. When used in a balanced and responsible way, the plant extracts can support biodiversity and maintain long-term soil health, unlike approaches that rely heavily on synthetic chemicals [41,42]. *Salvia* species are increasingly recognized as promising natural sources of antifungal compounds, owing to their richness in bioactive terpenoids, phenolics, and essential oils active against several important plant pathogens [43,44]. Different extracts from *Salvia* spp. have shown strong activity against fungi, including *Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Colletotrichum coccodes* [5,8]. Carnosic acid isolated from *S. somalensis* reported significant inhibition of mycelial growth, highlighting the potential of these extracts as an eco-friendly method to control crop diseases [5]. Importantly, whole plant extracts often retain strong biological activity due to synergistic interactions among multiple phytochemicals, which can enhance

efficacy compared to isolated compounds, making them particularly attractive for sustainable crop protection strategies [45]. In the present study, we investigated the chemical profile and antifungal activity of the methanolic extract of *S. somalensis*. LC-MS analysis revealed that the methanolic extract was dominated by terpenoids and flavonoids. The antifungal assays confirmed that *S. somalensis* possessed strong bioactivity against several phytopathogenic fungi. The methanolic extract from the in vivo plant exhibited strong inhibition against all tested fungi, particularly *C. coccodes* and *B. cinerea*. *Colletotrichum* species are notorious for causing anthracnose disease in different crops [46], and similar results were noted in a previous study using the dichloromethane surface extract of *S. somalensis* [5]. Related findings have been reported for *S. discolor* extracts against *B. cinerea* (77.1%), with mycelial growth inhibition at 1000 µg/mL [8], further confirming the antifungal potential of *Salvia* spp. A genus-level review of *Salvia* species also highlights repeated reports of substantial in vitro activity against *Botrytis* and *Colletotrichum* spp., supporting our observation that members of this genus are a reliable source of antifungal metabolites [47]. Methanolic extract of *S. somalensis* also showed substantial inhibition (>70%) against *F. oxysporum* and *R. solani*, consistent with results for the dichloromethane plant surface extract in earlier studies conducted by Iobbi et al. [5]. The observed antifungal activity could be broadly attributed to the combined presence of several major phytochemical classes in this extract. Abietane diterpenoids, including carnosol and rosmanol derivatives, a signature group in *Salvia* species, play critical defence and signalling roles and exhibit diverse bioactivities [48]. Among them, carnosic acid is well known for its antimicrobial and antioxidant properties [5,16,49,50]. Interestingly, despite its lower carnosic acid content, the methanolic extract exhibited antifungal activity comparable to that of the carnosic acid-rich dichloromethane plant surface extract previously characterized from the same species [5]. For instance, at 1000 µg/mL, the methanolic extract inhibited *C. coccodes* and *B. cinerea* by 86.3% and 84.3%, respectively, values that fall within or exceed the 67–85% inhibition range previously reported. These findings suggest that carnosic acid was not the sole determinant of the antifungal activity exhibited by the methanolic extract and that other metabolites may contribute substantially to its bioactivity. Based on the tentative metabolite annotation, phenolic acids such as rosmarinic and caffeic acids, together with flavonoids such as isorhamnetin, may be involved in the observed antifungal effects. Previous studies have shown that these classes of compounds can impair fungal growth through multiple mechanisms, including the disruption of cell membrane integrity and inhibition of key enzymatic processes [51,52]. However, as these metabolites were not quantified in the present study, their specific contribution to the observed bioactivity cannot be directly determined and therefore remains speculative. Further investigations involving their isolation, quantification, and evaluation in targeted bioassays will be required to elucidate their individual roles. While synergistic interactions among phytochemicals are plausible and have been proposed in the literature, direct synergy testing was not performed in the present study. Therefore, the observed activity is more cautiously attributed to the combined, potentially additive or synergistic, action of multiple compound classes rather than to any single constituent. In several broader screening studies, different plant methanolic extracts reached their highest inhibitory effects at screening concentrations of around 500–1000 µg/mL [53]. Moreover, reports on other botanicals used in food-poisoning assays (e.g., *Larrea tridentata*, *Rosmarinus officinalis*, *Salvia officinalis*) show comparable and slightly lower inhibitory profiles under similar conditions, further indicating that the potency of *S. somalensis* extract is consistent with promising leads in phytochemical screening studies [54,55].

Considering the promising antifungal activity exhibited by the methanolic extract, *S. somalensis* biomass and callus cultures were subsequently established to evaluate the potential of in vitro culture systems as alternative sources of bioactive metabolites. Plant in vitro cul-

tures are widely recognized as sustainable, scalable, and reproducible production platforms for specialized metabolites, allowing biomass generation under controlled conditions while minimizing the influence of seasonal and environmental variability. Furthermore, recent advances in elicitation strategies, metabolomics, and bioprocess optimization have enhanced the potential of these systems for the production of high-value phytochemicals [56]. The cultures were successfully maintained without evident physiological disorders, including hyperhydricity. This observation is consistent with previous studies on *Salvia* sp., where low BA concentrations promoted shoot proliferation without inducing hyperhydricity [26,32]. The micropropagated plant extract demonstrated moderate activity, indicating that while bioactive potential is retained post-micropropagation, potency may decline due to reduced production of surface-secreted terpenoids, particularly 4β,6β-dihydroxy-1α,5α(H)-guai-9-ene, teucladiol, 11-hydroxy-12-methoxyabieta-8,11,13-triene, and β-chaenocephalol, previously identified only in dichloromethane plant surface extracts [5]. These compounds are likely synthesized or accumulated in trichome-rich tissues and can require elicitation for sustained production in vitro; similar reductions in surface-localized metabolites following micropropagation have been noted in several Lamiaceae species [47,57]. Extracts from callus and elicitor-treated callus cultures showed comparatively low antifungal activity, likely reflecting the reduced accumulation of bioactive specialized metabolites commonly observed in undifferentiated plant tissues. Although elicitation can stimulate secondary metabolism, the biosynthetic potential of callus cultures often remains lower than that of differentiated tissues, resulting in limited biological activity [56].

LC-MS analysis revealed that the micropropagated biomass and callus cultures produced some compounds isolated from the dichloromethane plant surface extract [5]. LC-MS/MS profiling of other *Salvia* species under in vitro conditions identified flavonoids, terpenoids, and phenolic acids, highlighting the metabolic plasticity of cultured tissues [11,58]. Interestingly, certain sesquiterpenoids, namely (−)-7-*epi*-isojunenol, (+)-junenol, and (+)-*ent*-epicubenol, detected in the leaf surface of *S. somalensis* appeared only in micropropagated plants and in callus cultures treated with salicylic acid as an elicitor. Similar findings have been documented where abiotic elicitors such as salicylic acid and methyl jasmonate significantly enhanced secondary metabolite biosynthesis in medicinal plants by activating defence-related pathways [59,60]. Some metabolites, including betulinic acid, 7,13-*E*-labdadien-15-ol, 12-*O*-methylacetylcarnosate, 12-methylcarnosol, and brussonol, were exclusively synthesized in micropropagated plants, suggesting that differentiated tissues may drive their production. However, carnosic acid (**1**), 1β,6β-dihydroxy-4(14)-eudesmene, betulin, and uvaol were common across leaf surface [5], in vitro biomass, and callus (non-elicited) cultures.

HPLC–UV quantification revealed low amounts of carnosic acid in methanolic extracts of both in vivo and in vitro cultures. This contrasts with previous findings showing high carnosic acid levels (11.39%, *w*/*w*, of extract and 0.12%, *w*/*w,* of fresh biomass) in the dichloromethane surface extract of *S. somalensis* [5]. This discrepancy likely reflects the selectivity of dichloromethane extraction, which recovers lipophilic metabolites localized in glandular trichomes compared to the total methanolic extraction [61,62].

Although the antifungal activity observed at 1000 µg/mL is encouraging, it should be noted that this is a relatively high concentration for practical applications. Before considering *S. somalensis* extracts as alternatives to synthetic fungicides in crop protection, further studies are needed to determine minimum inhibitory concentrations, assess in vivo efficacy under field conditions, and evaluate formulation strategies that might improve bioavailability and reduce effective doses. These results should therefore be interpreted as proof-of-concept findings that justify further investigation, rather than as direct evidence of applicability. Given the rising incidence of fungicide resistance and environmental con-

cerns, *Salvia*-derived metabolites offer a promising option for integrated pest management, aligning with global trends toward sustainable agriculture [63,64].

5. Conclusions

A comprehensive chemical characterization of in vivo plants, micropropagated cultures, callus cultures, and elicitor-treated callus of *Salvia somalensis* revealed a wide variety of secondary metabolites, including sesquiterpenoids, triterpenoids, flavonoids, and diterpenoids. Antifungal tests demonstrated that methanolic extracts of *S. somalensis* exhibit broad-spectrum activity against the phytopathogenic fungi tested, with in vivo extracts showing the greatest efficacy, followed by those obtained from micropropagation. These results suggest that bioactivity is closely associated with tissue differentiation and metabolic composition, particularly the presence of surface-associated terpenoids. These findings are based on in vitro mycelial inhibition assays, and although synergistic interactions among phytochemicals are plausible, they remain to be experimentally confirmed through combinatorial bioassays. Future research should focus on semi-quantitative metabolite profiling, optimization of elicitor concentrations, and validation under field conditions to fully assess the potential of *S. somalensis* extracts as sustainable crop protection agents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy16141344/s1, Figure S1. In vitro biomass of *Salvia somalensis*; Figure S2. LC-MS analysis of extracts of *Salvia somalensis*; Figure S3. Representative mycelial growth inhibition of *Colletotrichum coccodes*; Figure S4. Representative mycelial growth inhibition of *Fusarium oxysporum*; Figure S5. Representative mycelial growth inhibition of *Rhizoctonia solani*; Figure S6. Representative mycelial growth inhibition of *Sclerotinia sclerotiorum*; Table S1. Compounds detected in *Salvia somalensis* micropropagated plant extracts (References [34–39] are cited in the Supplementary Materials); Table S2. Dose–response parameters obtained from four-parameter logistic fitting; Table S3. Dose–response parameters obtained from exponential saturation model fitting.

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