Article

Potential of *Salvia discolor* Extract Against Common Agricultural Pathogens †

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Abstract: Phytopathogenic fungi and bacteria pose a serious threat to global agriculture, leading to significant economic losses and potential health risks. Consequently, the search for natural alternatives to synthetic agrochemicals has garnered increasing scientific attention, with plant extracts emerging as promising environmentally friendly solutions. In this context, the surface extract of *Salvia discolor*, obtained using dichloromethane, was analyzed for its bioactive potential. Chemical profiling revealed a rich composition of terpenoids and flavonoids. The antimicrobial potential of the ground extract was evaluated against nine phytopathogenic fungi (*Alternaria solani*, *Botrytis cinerea*, *Colletotrichum lindemuthianum*, *Fusarium solani*, *Fusarium oxysporum* f. sp. *lactucae* race 1, *Phoma betae*, *Phaeomoniella chlamydospora*, *Pythium dissotocum*, and *Stemphylium* sp.), and two phytopathogenic bacteria (*Clavibacter michiganesis* subsp. *michiganesis* and *Pectobacterium carotovorum* subsp. *carotovorum*), selected from common pathogens of agricultural interest. Complete inhibition of *P. chlamydospora* at 1000 μg mL^{-1} and strong activity against *P. dissotocum*, *F. solani* and *B. cinerea* was observed, and low inhibition (<40%) against *C. lindemuthianum* and *F. oxysporum* f. sp. *lactucae* race 1. However, the extract showed promising results in the post-harvest protection of tomatoes against gray mold. Moderate antibacterial activity was seen against *C. michiganensis* subsp. *michiganensis*. These findings indicate that *S. discolor* extract has the potential to serve as an effective natural crop protection agent, though further optimization may be needed for broader application.

Keywords: *Salvia discolor*; terpenoids; flavonoids; phytopathogens; *Botrytis cinerea*; crop protection

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1. Introduction

Significant losses in the agricultural sector are caused by phytopathogenic fungi and bacteria, which harm food production and cause health problems [1,2]. The most prevalent fungal genera affecting crops on a global scale are *Alternaria*, *Botrytis*, *Fusarium*, *Pythium*, and *Stemphylium* [3–5]. The most problematic and widespread plant pathogenic bacteria mainly originate from Gram-negative genera, including *Xanthomonas*, *Pseudomonas*, *Xylella*, and *Erwinia*, and Gram-positive genera such as *Arthrobacter*, *Clavibacter*, *Curtobacterium*,

and *Rhodococcus* [6]. The presence of these organisms in agricultural systems poses a significant challenge to the implementation of effective pest management and crop protection strategies, resulting in considerable economic losses due to reduced yields and low-quality products [7–9]. Pathogenic fungal diseases account for approximately 65–70% of all crop losses caused by pathogens [10,11].

Chemical pesticides are commonly used for their effectiveness in reducing pest-related crop damage [12]. However, they pose severe health risks to humans, including fetal impairments and cancers, and are non-biodegradable, persisting in the environment for years [13,14]. The increased production of synthetic substances for agricultural use has underscored the need to develop sustainable disease control strategies that minimize risks to humans and the environment [15]. Research into natural products as potential fungicides and bactericides offers a promising, environmentally friendly alternative to mitigate the harmful effects of these pathogens and safeguard crop yield and quality. This approach could facilitate the identification of safer alternatives to traditional pesticides [16]. Plants produce various metabolites with bactericidal, fungicidal, insecticidal, and nematocidal properties to protect themselves against phytophagous organisms and diseases [17]. Several studies have demonstrated the potential of these natural substances to be integrated with traditional crop management practices, addressing the urgent need for sustainable agriculture, safe food production, and organic farming, which are advocated for by governmental bodies and major retailers [18–20].

The genus *Salvia* comprises nearly one thousand species of herbs, shrubs, and perennial plants, making it the largest genus within the Lamiaceae [21]. These plants are notably abundant in various natural compounds, including monoterpenoids, diterpenoids, phenolic acids, triterpenoids, flavonoids, and saccharides [22]. Historically, the *Salvia* genus has been used for diverse applications, such as in herbal beverages, culinary appetizers, and addressing ailments like colds, stomachaches, and gastrointestinal discomforts [23]. Furthermore, extensive research describes the biological activity of this genus's members, including their anticancer, antioxidant, antihypertensive, hypoglycemic, hypolipidemic, memory-enhancing, antiviral, antimicrobial, and phytotoxic properties [22,24–26]. Phytochemicals derived from *Salvia* have demonstrated significant potential in combating phytopathogens responsible for diseases and substantial economic losses in agricultural systems globally [27]. In particular, surface extracts play an important role in plant defense by providing toxic, antifeedant, antifungal and antibacterial protection against herbivores and pathogens, thanks to the secretion of defense compounds onto the cuticular layer [28,29], and lipophilic extracts of several *Salvia* species have shown efficacy against several plant pathogens [24–26]. Due to their natural properties, several sage-derived antifungal compounds have been classified as "generally recognized as safe" (GRAS) for food preservation and crop protection [30]. Despite the promising potential of natural products, there is still a considerable gap in understanding the full spectrum of bioactive compounds found in *Salvia* species and their efficacy against a wide range of plant pathogens [27].

Addressing this gap, our study seeks to characterize the phytochemical profile of the surface dichloromethane extract of *Salvia discolor* Kunth [31–33], and to evaluate its bioactivity against phytopathogens, thus offering valuable insights into the development of novel, sustainable crop protection strategies [34]. Nine phytopathogenic fungi (*Fusarium solani*, *Phoma betae*, *Phaeomoniella chlamydospora*, *Colletotrichum lindemuthianum*, *Fusarium oxysporum* f. sp. *lactucae* race 1, *Pythium dissotocum*, *Alternaria solani*, *Stemphylium* sp., and *Botrytis cinerea*), and two phytopathogenic bacteria (*Clavibacter michiganensis* subsp. *michiganensis* and *Pectobacterium carotovorum* subsp. *carotovorum*), selected from the more common pathogens of agricultural interest, were tested. These pathogens induce various diseases, ranging from anthracnose and wilt diseases to bacterial canker and soft rot, resulting in

more than 60% losses in agricultural production [10,35]. *Fusarium solani* is the main cause of root rot in legumes. This pathogen significantly affects the production of several legumes and vegetables, including beans, soybeans, peas, lentils, tomatoes, and cucurbits [36,37]. *Phoma betae* is known to cause leaf spots and root rot disease in beets and various other plants [36,38]. *Phaeomoniella chlamydospora* is identified as the principal fungal pathogen causing vascular diseases in grapevines, particularly within the Esca complex and Petri disease [39]. *Colletotrichum lindemuthianum* is classified as a hemibiotrophic fungus responsible for anthracnose, affecting common beans (*Phaseolus vulgaris*) and other *Phaseolus* species [40]. *Fusarium oxysporum* is a global ascomycete fungus, usually saprophytic and nonpathogenic, commonly found in soil. Certain strains cause wilt disease by invading the plant's vascular system, resulting in severe wilting and often the death of the host plants [41,42]. *Pythium dissotocum* is a major pathogen responsible for seedling damping off and causes a range of severe diseases in various plants, affecting everything from young annual vegetable seedlings to mature forest trees [43,44]. *Alternaria solani,* a necrotrophic fungus, causes early bird disease in tomatoes, significantly impacting stems, foliage, and fruits, leading to substantial economic losses [45]. *Stemphylium* sp. causes gray leaf spots in tomato crops, resulting in significant defoliation and yield losses. Symptoms, visible on both seedlings and plants, manifest as circular to elongated dark specks on leaves, enlarging and becoming gray with age [46]. *Botrytis cinerea* affects numerous plant species, including grapevines, fruits, vegetables and ornamentals, and can infect almost all plant parts, from stems to leaves, flowers, and fruits, leading to gray mold disease [47,48]. *Clavibacter michiganensis* subsp. *michiganensis* is a Gram-positive bacterium that causes bacterial canker in tomatoes, which is particularly destructive in greenhouse conditions [49]. *Pectobacterium carotovorum* subsp. *carotovorum*, a Gram-negative pathogen, induces severe soft rot in various economically important vegetables, including carrots, cabbage, cucumbers, eggplants, garlic, onions, peppers, potatoes, radishes, sweet potatoes, pumpkins, and tomatoes [50].

Salvia discolor is cultivated as an ornamental in the Ligurian region (Italy) (information about the species can be found in the Supplementary Materials). Ornamental plant cultivation produces waste biomass, and this study is part of our research on the potential extractive use of waste biomass from local crops in the vision of sustainable agriculture [51,52].

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations, LC, TLC and MPLC chromatography, semi-preparative HPLC, and NMR experiments were performed as previously described [53,54].

MPLC chromatography was carried out using normal phase Si60 cartridges Supervarioflash and LiChroprep RP-18 (40–63 µm) (Merck, Darmstadt, Germany) in conjunction with a spot liquid chromatography system (Armen Instrument, Saint Ave, France). For TLC analysis, Silica gel 60 F254-coated aluminum sheets (Merck, dimensions: 20 × 20 cm, layer thickness: 0.2 mm) were used. The mobile phase composition was $CHCl_3/CH_3OH/HCOOH$ (10:0.5:0.1), and spot visualization involved spraying with 50% H_2SO_4, followed by gentle heating. Flash chromatography was performed over an Isolera® Biotage® purification system (Biotage, Uppsala, Sweden) equipped with a Biotage flash SNAP KP-Sil column of 50 g, a flow rate of 35 mL/min, and a collection volume of 9 mL. Fractions were collected according to UV absorbance at 254 and 280 nm.

Semi-preparative HPLC employed 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 column, Symmetry Prep C18, 7.8 × 300 mm i.d., with a 7 µm particle size (Waters), was used at room temperature, with

a flow rate of 1.03 mL min^{-1}. The sample loop was 100 μL, and the eluents were A: H_2O, B: CH_3OH; gradient: B 50% at time 0, B 100% at 61 min, B 100% at 75 min, B 100% at 100 min. A Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) coupled with a Bruker 5 mm TCI CryoProbe at 300 K was used for NMR investigations. Every 2D NMR spectrum was obtained in CDCl3 (99.8%, Sigma-Aldrich, Milano, Italy), and the HSQC, COSY, HMBC, and ROESY spectra were obtained employing conventional pulse sequences and phase cycling. UXNMR software was employed to process the NMR data. t_{mix} = 400 ms was used to acquire the ROESY spectra.

HRESIMS data were obtained in both the positive and negative ion mode on a Q Exactive Plus mass spectrometer, Orbitrap-based FT-MS system, equipped by an ESI source (Thermo Fischer Scientific Inc., Bremen, Germany). Following the manufacturer's instructions, the Orbitrap mass analyzer was calibrated with a combination of caffeine, sodium dodecyl sulfate, sodium taurocholate, methionine-arginine-phenylalanine-alanine-acetate (MRFA), and Ultramark 1621. Information was collected and analyzed employing the software supplied by the manufacturer.

2.2. Plant Material

The fresh aerial parts of *S. discolor* were obtained from Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Sanremo (IM), Italy. The plant material was identified by Dr. Andrea Copetta and a voucher specimen (HMGBH.e/7290.2024.001) was deposited at Herbarium of Giardini Botanici Hanbury (La Mortola, Ventimiglia, Italy).

2.3. Chemicals

Extraction and purification solvents were acquired from VWR International Srl, (Milano, Italy). $CDCl_3$ (99.8%) was acquired from Sigma-Aldrich (Milano, Italy).

2.4. Extraction and Isolation

1.2 Kg of freshly harvested aerial parts of *S. discolor* were briefly immersed in CH_2Cl_2 for 20 s [26] affording 53 g of dry residue. This extract underwent *n*-hexane partitioning, yielding an *n*-hexane-soluble (26.6 g) and an *n*-hexane-insoluble portion (23.2 g).

The *n*-hexane-soluble portion was separated by CC on silica gel (MPLC; monitoring by TLC), eluted with mixtures of *n*-hexane/$CHCl_3$/CH_3OH (from 1:1:0 to 0:0:1, 2.28 L) to afford 37 fractions (I_1–I_{37}). Fraction I_{26} (130.0 mg), eluted with a mixtures of $CHCl_3$/CH_3OH from 1.47 to 1.56 L, was purified by semi-preparative RP-HPLC, affording **1** (2.9 mg; t_R = 75.5 min).

The *n*-hexane-insoluble extract was then treated with aqueous CH_3OH (80%) to obtain a methanol-soluble fraction (7.2 g), and a methanol-insoluble fraction, mainly constituted by triterpene compounds, particularly ursolic and oleanolic acids (10.2 g). The methanol-soluble fraction was separated by CC on silica gel (MPLC; monitoring by TLC), eluted with mixtures of *n*-hexane/$CHCl_3$/CH_3OH (from 1:1:0 to 0:0:1, 1.65 L) to afford 11 fractions (II_1–II_{11}). Fraction II_5 (1.2 g), eluted with $CHCl_3$ from 0.51 to 0.57 L, was re-chromatographed on silica gel (MPLC; monitoring by TLC) eluted with mixtures of *n*-hexane/$CHCl_3$/CH_3OH (from 1:1:0 to 0:0:1, 1.68 L), affording 17 fractions (III_1–III_{17}). Fraction III_8 (120.0 mg), eluted $CHCl_3$ from 0.81 to 0.84 L, was further purified by semi-preparative RP-HPLC, affording **2** (0.7 mg; t_R = 63.0 min) and **3** (3.1 mg; t_R = 86.5 min). Fraction III_{10} (175.2 mg), eluted with $CHCl_3$ from 0.87 to 0.90 L, was further purified by semi-preparative RP-HPLC, affording **4** (2.6 mg; t_R = 52.0 min).

Fraction II_6 (741.0 mg), eluted with $CHCl_3$ from 0.57 to 0.60 L, was subjected to Isolera Biotage CC eluted with *n*-hexane/ethyl acetate (from 72:28 to 28:72, 0.89 L), affording 7 fractions (IV_1–IV_7). Fraction IV_4 (128.7 mg), eluted with *n*-hexane/ethyl acetate 58:42 from 0.4 to 0.6 L, was purified by semi-preparative RP-HPLC affording **5** (1.0 mg; t_R = 37.5 min).

Fraction II_7 (0.7 g), eluted with $CHCl_3$ from 0.60 to 0.74 L, after MPLC separation, afforded 12 fractions (V_1–$V1_2$). Fraction V_6 (103.5 mg), eluted with $CHCl_3$ from 0.87 to 0.93 L, was purified by semipreparative RP-HPLC affording **6** (1.0 mg; t_R = 35.1 min) and **7** (2.0 mg, t_R = 60.5).

Fraction II_9 (1.2 g), eluted with $CHCl_3/CH_3OH$ 95:05 from 1.14 to 1.20 L, was subjected to Isolera Biotage CC eluting with *n*-hexane/ethyl acetate (from 1:1 to 0:1, 1.02 L), affording 12 fractions (VI_1–VI_{12}). Fraction VI_6 (131.7 mg), eluted with *n*-hexane/ethyl acetate 87:13 from 0.24 to 0.30 L, was purified by semipreparative RP-HPLC affording **8** (1.4 mg; t_R = 51.5 min) and **9** (1.2 mg; t_R = 69.5 min). Fraction VI_7 (159.8 mg), eluted with *n*-hexane/ethyl acetate 87:13 from 0.30 to 0.39 L, was purified by semi-preparative RP-HPLC affording **10** (3.7 mg; t_R = 60.0 min).

The purity of the isolated compounds was confirmed by TLC and HPLC (purity > 95% for compounds **1–8**. Compound **9** showed purity > 90%).

2.5. Microorganisms

Phytopathogenic fungi species *A. solani*, *C. lindemuthianum*, *F. solani*, *F. oxysporum* f. sp. *lactucae* race 1, *P. betae*, *P. chlamydospora*, *P. dissotocum*, and *Stemphylium* sp. were isolated from symptomatic plants of *Solanum tuberosum*, *Vitis vinifera, Phaseolus vulgaris*, *Curcurbita pepo*, *Lactuca sativa*, *Spinacia oleracea*, *Apium graveolens*, and *S. lycopersicum*, respectively, at CeRSAA (Albenga (SV), Italy). Four strains of *B. cinerea* were isolated from *Lavandula angustifolia* (strain 1), *S. lycopersicum* (strain 2), *Viburnum opulus* (strain 4), and *Fragaria x ananassa* (strain 11). Bacterial strains of *C. michiganensis* subsp. *michiganensis* and *P. carotovorum* subsp. *carotovorum* were isolated from symptomatic plants of *Solanum lycopersicum* and *Brassica oleracea*, respectively.

2.6. Poisoned Food Technique

The antifungal activity of the dichloromethane surface extract of *S. discolor* was evaluated against several plant pathogenic fungi using the poisoned food technique [55]. The inhibitory effect on mycelium growth of *A. solani*, *B. cinerea*, *C. lindemuthianum*, *F. solani*, *F. oxysporum* f.sp. *lactucae* race 1, *P. betae*, *P. chlamydospora*, *P. dissotocum*, and *Stemphylium* sp. has been tested. Culture medium was prepared by autoclaving 39 g of potato dextrose agar (Merck) in 1 L of distilled water at 121 °C for 15 min. After cooling to 50 °C, the crude surface extract [dissolved in 1% *v*/*v* DMSO (Sigma Aldrich, St. Louis, MO, USA)] was added to the medium at concentrations of 5, 100, 250, 500, 750, and 1000 μg mL^{-1}. The range of extract concentrations (5–1000 μg/mL) was selected based on previously published studies on the antifungal activity of plant extracts, which commonly utilized similar concentration ranges to evaluate the effect at different doses [52,56,57]. The medium was then supplemented with streptomycin sulfate (5 μg mL^{-1}) and solidified in 90 mm diameter Petri dishes. Four synthetic fungicides, Ortiva® (Syngenta, Milano, Italy) (azoxystrobin 23%) corresponding to a concentration of azoxystrobin 250 μg mL^{-1}, Switch® (Syngenta, Milano, Italy) (cyprodinil 37% + fludioxonil 25%) corresponding to a concentration of cyprodinil 375 μg mL^{-1} and fludioxonil 250 μg mL^{-1}, Score® 25 EC (Syngenta, Milano, Italy) (difenoconazole 23.6%) corresponding to a concentration of difenoconazole 250 μg mL^{-1}, and Ridomil Gold®R WG (metalaxyl-m 2%+ copper 14.19%) corresponding to a concentration of metalaxyl-m 20 μg mL^{-1} and copper 141.9 μg mL^{-1}, were included as controls at the manufacturer-recommended concentrations. Sterile agar plugs (approximately 2 × 2 mm) of the test fungi were placed centrally on the prepared media in Petri dishes, which were incubated in the dark at 25 °C. Each concentration of the extract was tested in triplicate, with additional controls consisting of media treated with 1% *v*/*v* DMSO and untreated media. The diameter of fungal growth was measured when the mycelium

fully colonized the untreated plate. The inhibition percentage of growth, compared to the untreated control, was calculated using the formula $I = [(C - T)/C] \times 100$, where I is the inhibition percentage, C and T are the mean diameters (cm) of the fungal colonies in the control (untreated) plate and the treated plate, respectively [58]. The experiments were repeated three times, yielding consistent results across all trials.

2.7. Efficacy of S. discolor Extract Against Post-Harvest Diseases in Tomato Fruit

Based on the in vitro results, the concentrations of 100, 500, and 1000 μg mL^{-1} of dichloromethane extract were selected as the optimal levels for subsequent in vivo experiments on tomato fruits against *B. cinerea*. The experiments were carried out according to the method described by Liu and co-workers [59] with a few modifications. Healthy tomatoes var. Maraskino (Seminis, Vegetables, Bayer, Leverkusen, Germany) were obtained from organic local farms in Albenga (SV) and washed with tap water to remove surface dust. The fruit was then disinfected by immersion in a 1% v/v sodium hypochlorite solution for 20 min, followed by two rinses with distilled water and air drying at 25 ± 2 °C. Tomato fruits were wounded at the equator using a sterile scalpel, creating incisions 3 mm deep and 3 mm wide. These wounds served as the sites for applying the dichloromethane extract and subsequent inoculation with *B. cinerea*. Preventive and curative treatments were applied during the tests. For the preventive treatments, tomato fruits were first treated with dichloromethane extract dissolved in 1% v/v DMSO at concentrations of 100, 500, and 1000 μg mL^{-1}, applied using a nebulizer. Control treatments included 1% *v/v* DMSO and distilled water, while Teldor® (Bayer Cropscience s.r.l, Milano, Italy) (fenhexamid 42.7%) corresponding to a concentration of fenhexamid 750 μg mL^{-1}, and 1% *v/v* peracetic acid served as reference treatments. After 4 h, *B. cinerea* conidial suspension (5.7×10^4 spores mL^{-1}) was applied to the fruits. For the curative treatments, the process was reversed: tomato wounds were first inoculated with the *B. cinerea* conidial suspension, followed by a 10 min waiting period. The dichloromethane extract, the control, and the reference solutions were then applied. Treated fruits were placed in plastic boxes ($200 \times 130 \times 50$ mm) and maintained at high relative humidity (~95%). Fruits treated with extract and positive and negative controls were kept in a climatic chamber at 18 ± 2 °C with a 14:10 dark: light ratio. The percentage of decay incidence caused by *B. cinerea* was assessed 7 days post-inoculation for both treatments. Each treatment was conducted in triplicate, and the experiment was repeated three times, for a total of 27 fruits per treatment [60].

2.8. Antibacterial Assay

To evaluate the antibacterial effect of *S. discolor* dichloromethane extract, two phytopathogenic bacteria (*C. michiganensis* subsp. *michiganensis* and *P. carotovorum* subsp. *carotovorum*) were utilized. A sterile stock solution of the dichloromethane extract in dimethyl sulfoxide (DMSO) was prepared and stored at −20 °C. This stock solution was subsequently diluted 1:10 with Mueller–Hinton broth medium (Merck-Millipore, Burlington, MA, USA), resulting in a final concentration of 2000 μg mL^{-1}, which is twice the highest concentration tested. A sample of 100 μL of this solution was transferred to the wells in the first column of a microtiter plate. Similarly, sterile stock solutions of streptomycin sulfate (VWR Life Science, Radnor, PA, USA), bacitracin (Sigma-Aldrich, Milano, Italy), and ampicillin (Sigma-Aldrich, Milano, Italy) were prepared in DMSO and diluted 1:10 with Mueller–Hinton broth, achieving a concentration of 64 μg mL^{-1}, double the highest test concentration. Each antibiotic solution (100 μL) was also transferred to the wells of the first column of a microtiter plate. One-day-old bacterial cultures were prepared by diluting them in Buffered Peptone Water (VWR Life Science, Radnor, PA, USA) until the suspension reached 0.5 on the McFarland scale. This bacterial suspension was then further diluted

to 1/150 in Mueller–Hinton broth (Merck-Millipore, Burlington, MA, USA), achieving a final concentration of approximately 5×10^5 cells mL^{-1}. A microdilution procedure was used to determine the MIC (minimum inhibitory concentration value) reported by the Clinical and Laboratory Standards Institute using Mueller–Hinton broth as the test medium [61]. In summary, a 96-well microplate was prepared with 2-fold serial dilutions of the dichloromethane extract (0.976 to 1000 μg mL^{-1}), and 50 μL of the inoculum generated as described above was introduced to corresponding amounts of various concentrations of the dichloromethane extract. At the same time, the inoculum was added to equivalent volumes of ampicillin and streptomycin sulfate spread over a 96-well microplate and prepared from 2-fold serial dilutions ranging from 0.0312 to 32 μg mL^{-1}. The activity of DMSO was tested in the last second row, ranging from 0.0488 to 50 μL mL^{-1}. Not-inoculated sterile Mueller–Hinton broth (MHB) medium served as a blank control in the final row, whereas drug-free wells were used in the final column to confirm bacterial growth. Bacterial growth was assessed by visual inspection of turbidity in the wells. The minimum inhibitory concentration (MIC) was determined by measuring the lowest concentration of chemicals that prevented observable growth after a 24 h incubation period at 35 °C. Each MIC determination was conducted in triplicate [61].

2.9. Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate the effects of treatments, followed by Tukey's HSD test for post hoc mean separation at a significance level of $p \leq 0.05$. Each assay included three replicates per treatment. The assumptions underlying ANOVA were verified and met. Descriptive statistics and visual inspection of the data suggested that the data were approximately normally distributed. Given the small sample size per group, visual assessment was considered more appropriate than formal normality tests, which can be unreliable with very small samples. Homogeneity of variances was assessed with Levene's test. The version of the test based on the median, which is more robust to non-normality and outliers, indicated that the assumption of equal variances was met. This supports the validity of the ANOVA results. ANOVA was performed using Statistica software, version 8.0 (StatSoft Inc., Tulsa, OK, USA). Generalized linear models (GLMs) were constructed using the lme4 package version 1.1.37 in R [62,63] to investigate the percentage of inhibition, the administered dose, and fungal species identity. Various model configurations were tested, including the additive effect of the predictors and the interaction between the variables. In addition, the effect of the quantitative variable dose was evaluated in its original form as well as in logarithmic, second-degree, and third-degree polynomial transformations. The model with the highest coefficient of determination (R^2) and statistical significance was selected for further analysis. Predicted inhibition values were computed using the ggpredict function from the ggeffects package [64]. For each fungal species, the median effective dose (ED_{50}) [65] and corresponding 95% confidence intervals were estimated using MATLAB R2024b (Microsoft, Redmond, WA, USA). Additionally, a third-degree polynomial was fitted to the data using the polyfit function, with absolute dose values on the x-axis and inhibition percentages on the y-axis. This polynomial degree was chosen to capture non-linear trends while minimizing the risk of overfitting. The resulting equation was solved using the roots function, and 95% confidence intervals were calculated using the polyconf function. Model fit quality was evaluated using the coefficient of determination (R^2).

3. Results

3.1. Chemical Analysis

The dichloromethane extract obtained from the plant surface of *S. discolor* afforded some known compounds, gardaubryone C (**1**) [66], salvigenin (**2**) [67], salvilymitol (**3**) [68], eupatorin (**4**) [69], carnosol (**5**) [70], an *ent*-clerodane diterpene dilactone, rhynchosperin A (**6**) [71,72], ermanin (**7**) [73], kaempferide (**8**) [74], divinatorin A (**9**) [75], and patagonic acid (**10**) [76] (Figure 1), which were identified through NMR analysis (Figures S1–S10, Supplementary Materials), including ^{1}H and ^{13}C NMR, TOCSY, HSQC, and HMBC experiments, and HRESIMS data.

Figure 1. Compounds isolated from *S. discolor*.

3.2. Antifungal Assays

The antifungal efficacy of *S. discolor* dichloromethane extract was assessed in vitro at various concentrations against nine phytopathogenic fungi (*A. solani*, *B. cinerea*, *C. lindemuthianum*, *F. solani*, *F. oxysporum* f. sp. *lactucae* race 1, *P. betae*, *P. chlamydospora*, *P. dissotocum*, and *Stemphylium* sp.). The activity was compared with four synthetic fungicides and expressed as a percentage of inhibition of mycelial growth. The percentage of growth inhibition was determined by comparing mycelial growth in the presence

of different concentrations of the tested compounds with growth in the control condition, where no compounds were applied. Representative figures illustrating the inhibition of fungal mycelial growth after inoculation onto a medium containing the extract are provided in the Supplementary Materials (Figures S11–S14). Complete inhibition of *P. chlamydospora* was observed at the extract concentration of 1000 μg mL^{-1} (Table 1), statistically comparable to the standard fungicides Switch® (cyprodinil 375 μg mL^{-1} and fludioxonil 250 μg mL^{-1}), Score® 25 EC (difeconazole 250 μg mL^{-1}), and Ridomil Gold®R WG (metalaxyl-m 20 μg mL^{-1} and copper 141.9 μg mL^{-1}). At a lower concentration, 100 μg mL^{-1}, a 59% inhibition rate was recorded, and at higher concentrations (250, 500, and 750 μg mL^{-1}) 77.1%, 80.9%, and 88.6% inhibition rates, respectively, were observed (Table 1).

At the concentration of 1000 μg mL^{-1} the extract achieved a 91.4% inhibition rate against *F. solani*, a better result than with Score® 25 EC (difenoconazole, 250 μg mL^{-1}) and Ridomil Gold® WG (metalaxyl-m 20 μg mL^{-1} and copper 141.9 μg mL^{-1}), but lower than that obtained with Ortiva® (azoxystrobin 250 μg mL^{-1}). At the concentration of 1000 μg mL^{-1}, the extract achieved a 91.4% inhibition rate against *P. dissotocum*, a better result than with Score® 25 EC (difenoconazole, 250 μg mL^{-1}), but lower than other fungicides. The extract exhibited 70.5% inhibition against *P. betae* at a concentration of 1000 μg mL^{-1}, though this was not comparable to synthetic fungicides. Low inhibitory effects were observed against *A. solani, Stemphylium* sp., and *B. cynerea*, and the least inhibition was observed against *C. lindemuthianum* and *F. oxysporum* f. sp. *lactucae* race 1, with less than 40% inhibition across the tested concentrations.

The GLM highlighted statistically significant effects for the interaction between species and dose, albeit with differences between species in the estimated effect. In particular, the treatment was more effective on *P. chlamydospora* (estimated effect of dose = 0.098) and *F. solani* (estimated effect of dose = 0.084), while it was very low for other species, including *C. lindemuthianum* (estimated effect of dose = 0.012) and *F. oxysporum* f. sp. *lactucae* (estimated effect of dose = 0.004) (Figure S15, Supplementary Material).

Furthermore, ED_{50}, the dose required to inhibit 50% of fungal growth, was calculated (Table 2). Among the tested fungi, *P. chlamydospora* was the most sensitive to the extract, with the lowest ED_{50} value of 71.73 μg mL^{-1}. Antifungal activity was also observed against *F. solani* (ED_{50} 185.55 μg mL^{-1}), and lower activity was observed against *P. betae* (ED_{50} 277.41 μg mL^{-1}) and *P. dissotocum* (ED_{50} 530.82 μg mL^{-1}). ED_{50} values for *C. lindemuthianum*, *F. oxysporum* f. sp. *lactucae* (race 1), and *B. cynerea* could not be determined, indicating very limited efficacy.

A further evaluation of the extract's efficacy against three additional strains of *B. cinerea* from different diseased crops is presented in Table 3. For strain 1 (*L. angustifolia*), only 20% inhibition was observed at the highest extract concentration. In field conditions, crops like *L. angustifolia* and *V. opulus* typically receive fewer treatments due to economic constraints, making frequent applications impractical. In contrast, for strain 2 (*S. lycopersicum*) and strain 11 (*F. x ananassa*), inhibition rates at 750 and 1000 μg mL^{-1} were significantly higher than those obtained with Ridomil Gold®R WG (metalaxyl-m 20 μg mL^{-1} and copper 141.9 μg mL^{-1}) and Ortiva® (azoxystrobin 250 μg mL^{-1}). These strains have a higher potential for developing resistance due to their origin from crops subjected to high-load treatments. The inhibition levels for all tested *B. cinerea* strains were still lower compared to those achieved with Switch® (cyprodinil 375 μg mL^{-1} and fludioxonil 250 μg mL^{-1}).

Table 1. Percentage of inhibition of mycelial growth of nine phytopathogenic fungi treated with different concentrations of *S. discolor* dichloromethane extract compared to four standard fungicides [1].

Treatment	Concentration ($\mu g\ mL^{-1}$)	*Fusarium solani*	*Phoma betae*	*Phaeomoniella chlamydospora*	*Colletotrichum lindemuthianum*	*Fusarium oxysporum* f. sp. *lactucae* Race 1	*Pythium dissotocum*	*Alternaria solani*	*Stemphylium* sp.	*Botrytis cinerea* (Strain 4)
Crude extract	5	6.67 ± 1.55^{b}	11.42 ± 0.04^{b}	31.42 ± 0.04^{b}	5.71 ± 0.03^{b}	0.95 ± 1.64^{a}	16.19 ± 1.64^{b}	2.85 ± 0.04^{b}	4.74 ± 1.56^{b}	4.76 ± 1.63^{b}
Crude extract	100	43.81 ± 1.60^{c}	28.57 ± 0.03^{c}	59.04 ± 1.60^{c}	15.23 ± 1.56^{c}	8.57 ± 0.04^{b}	27.61 ± 1.60^{c}	19.40 ± 1.59^{c}	28.57 ± 0.04^{c}	14.28 ± 0.01^{c}
Crude extract	250	54.29 ± 0.04^{d}	48.57 ± 0.04^{d}	77.14 ± 0.04^{d}	18.09 ± 1.63^{d}	8.57 ± 0.03^{b}	27.61 ± 1.55^{c}	29.52 ± 1.55^{d}	28.57 ± 0.04^{c}	18.09 ± 1.61^{d}
Crude extract	500	71.43 ± 0.04^{e}	57.14 ± 0.03^{e}	80.86 ± 1.61^{e}	22.85 ± 0.04^{e}	11.42 ± 0.04^{c}	51.42 ± 0.02^{d}	37.14 ± 0.03^{e}	40.0 ± 0.03^{d}	34.28 ± 0.04^{e}
Crude extract	750	80.00 ± 0.03^{f}	57.14 ± 0.03^{e}	88.57 ± 0.04^{f}	28.54 ± 0.03^{f}	23.80 ± 0.03^{d}	64.74 ± 1.59^{e}	40.00 ± 0.04^{f}	51.42 ± 0.02^{e}	37.14 ± 0.02^{f}
Crude extract	1000	91.43 ± 0.04^{g}	70.51 ± 0.02^{f}	100 ± 0.00^{g}	31.42 ± 0.04^{g}	28.57 ± 0.04^{e}	91.42 ± 0.0^{fg}	55.23 ± 1.63^{g}	57.14 ± 0.01^{f}	42.54 ± 0.02^{g}
Cyprodinil + Fludioxonil	375 + 250	100 ± 0.00^{i}	100 ± 0.00^{i}	100 ± 0.00^{g}	100 ± 0.00^{j}	91.42 ± 0.04^{h}	100 ± 0.00^{g}	100 ± 0.00^{h}	100 ± 0.00^{g}	100 ± 0.00^{j}
Metalaxyl-m + copper	20 + 141.9	97.14 ± 0.03^{h}	94.28 ± 0.00^{h}	100 ± 0.00^{g}	100 ± 0.00^{j}	88.57 ± 0.04^{g}	100 ± 0.00^{g}	100 ± 0.00^{h}	100 ± 0.00^{g}	97.14 ± 0.00^{ij}
Difenoconazole	250	97.14 ± 0.03^{h}	100 ± 0.00^{i}	100 ± 0.00^{g}	82.85 ± 0.0^{i}	88.57 ± 0.04^{g}	84.66 ± 1.63^{f}	100 ± 0.00^{h}	98.08 ± 0.02^{g}	93.34 ± 0.02^{i}
Azoxystrobin	250	80.01 ± 0.04^{f}	88.57 ± 0.03^{g}	80.00 ± 0.04^{e}	42.85 ± 0.04^{h}	55.23 ± 0.03^{f}	100 ± 0.00^{g}	28.57 ± 0.04^{d}	100 ± 0.00^{g}	65.71 ± 0.03^{h}
DMSO	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.0 ± 0.000^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

[1] Data expressed as mean inhibition percentages ± standard deviation. Superscript letters denote significant differences at $p \leq 0.05$, as determined by Tukey's test.

Table 2. Median effective dose (ED_{50}) of the extract against the selected fungal species.

Fungal Species	Equation	ED_{50} (μg mL^{-1}) [a]	R^2
Fusarium solani	$y = 2.291 \times 10^{-7}x^3 - 0.0004312x^2 + 0.2835x + 10.78$	185.55 (127.11–263.02)	0.97262
Phoma betae	$y = 2.229 \times 10^{-7}x^3 - 0.0004022x^2 + 0.24x + 9.606$	277.41 (258.11–299.41)	0.99896
Phaeomoniella chlamydospora	$y = 2.693 \times 10^{-7}x^3 - 0.0004788x^2 + 0.278x + 32.43$	71.73 (45.63–100.84)	0.98336
Colletotrichum lindemuthianum	$y = 2.693 \times 10^{-7}x^3 - 0.0004788x^2 + 0.278x + 32.43$	NA [b]	0.97038
Fusarium oxysporum f. sp. *lactucae* race 1	$y = 2.693 \times 10^{-7}x^3 - 0.0004788x^2 + 0.278x + 32.43$	NA [b]	0.94038
Pythium dissotocum	$y = 3.037 \times 10^{-8}x^3 - 2.067 \times 10^{-5}x^2 + 0.06409x + 17.26$	530.82 (430.21–623.86)	0.98556
Alternaria solani	$y = 1.812 \times 10^{-7}x^3 - 0.0003042x^2 + 0.1756x + 2.757$	945.87 (923.57–965.98)	0.99751
Stemphylium sp.	$y = 8.645 \times 10^{-8}x^3 - 0.0001666x^2 + 0.1295x + 8.679$	803.63 (412.44 [c])	0.93221
Botrytis cinerea (strain 4)	$y = 8.645 \times 10^{-8}x^3 - 0.0001666x^2 + 0.1295x + 8.679$	NA [b]	0.98048

[a] Values in parenthesis are for fiducial limits (min.–max.). [b] The ED_{50} could not be determined when the fitted polynomial did not intersect with the 50% inhibition level within the experimental dosage range. [c] The upper bound of the confidence interval is missing because the corresponding root falls outside the range of the dosage data.

Table 3. Percentage of inhibition of mycelial growth of four strains of *Botrytis cinerea* treated with different concentrations of *S. discolor* dichloromethane extract compared to four standard fungicides [1].

Treatment	Concentration μg mL^{-1}	*Botrytis cinerea* (Strain 4)	*Botrytis cinerea* (Strain 1)	*Botrytis cinerea* (Strain 2)	*Botrytis cinerea* (Strain 11)
Crude extract	5	4.76 ± 1.63^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Crude extract	100	14.28 ± 0.01^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.71 ± 0.00^{b}
Crude extract	250	18.09 ± 1.61^{d}	2.85 ± 0.00^{a}	2.85 ± 0.00^{b}	5.71 ± 0.00^{b}
Crude extract	500	34.28 ± 0.04^{e}	8.57 ± 0.00^{a}	48.57 ± 0.04^{c}	37.14 ± 0.04^{c}
Crude extract	750	37.14 ± 0.02^{f}	8.57 ± 0.04^{a}	65.73 ± 0.03^{e}	71.42 ± 0.01^{f}
Crude extract	1000	42.54 ± 0.02^{g}	20.0 ± 0.03^{b}	74.97 ± 0.02^{f}	77.14 ± 0.04^{g}
Cyprodinil + Fludioxonil	375 + 250	100 ± 0.00^{j}	100 ± 0.00^{e}	100 ± 0.00^{g}	100 ± 0.00^{h}
Metalaxyl-m + copper	20 + 141.9	97.14 ± 0.00^{ij}	68.56 ± 0.02^{d}	58.19 ± 0.03^{d}	59.04 ± 1.59^{e}
Difenoconazole	250	93.34 ± 0.02^{i}	100 ± 0.00^{e}	100 ± 0.00^{g}	100 ± 0.00^{h}
Azoxystrobin	250	65.71 ± 0.03^{h}	45.71 ± 0.04^{c}	48.57 ± 0.00^{c}	51.4 ± 0.00^{d}
DMSO	10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

[1] Data expressed as mean inhibition percentages ± standard deviation. Superscript letters denote significant differences at $p \leq 0.05$, as determined by Tukey's test.

ED_{50} values (Table 4) revealed considerable variation in sensitivity among the tested *B. cinerea* strains. Strain 2 was the most responsive, with the lowest ED_{50} value of 566.10 μg mL^{-1}. Strain 11 showed a moderate susceptibility, with ED_{50} 583.20 μg mL^{-1}. Strains 4 and 1 showed substantial resistance or insensitivity to the extract.

Table 4. Median effective dose (ED_{50}) of the extract against *Botrytis cinerea* selected strains.

Fungal Species	Equation	ED_{50} (μg mL^{-1}) [a]	R^2
Botrytis cinerea (strain 4)	$y = 1.436 \times 10^{-8}x^3 - 5.365 \times 10^{-5}x^2 + 0.07685x + 4.984$	NA [b]	0.98048
Botrytis cinerea (strain 1)	$y = 3.963 \times 10^{-8}x^3 - 4.762 \times 10^{-5}x^2 + 0.0287x - 1.159$	NA [b]	0.95867
Botrytis cinerea (strain 2)	$y = -2.678 \times 10^{-7}x^3 + 0.0003832x^2 - 0.0418x - 0.5586$	566.10 (496.26–641.74)	0.97996
Botrytis cinerea (strain 11)	$y = -2.617 \times 10^{-7}x^3 + 0.0004002x^2 - 0.06393x + 3.087$	583.20 (540.15–627.90)	0.99245

[a] Values in parenthesis are for fiducial limits (min.–max.). [b] The ED50 could not be determined when the fitted polynomial did not intersect with the 50% inhibition level within the experimental dosage range.

3.3. Effect of the Dichloromethane Extract Against Post-Harvest Diseases in Tomato Fruit

The effects of the *S. discolor* extract and of reference controls varied between curative and preventive treatments against decay incidence caused by *B. cinerea* (Figure 2). Teldor®

(fenhexamid 750 µg mL^{-1}) was ineffective when used as a curative treatment but achieved 100% activity as a preventive treatment. Peracetic acid showed potent efficacy in the curative treatment, while its ability to control decay incidence in the preventive treatment was moderate. The dichloromethane extract at a concentration of 1000 µg mL^{-1} significantly protected tomatoes from gray mold disease in both preventive and curative applications. Interestingly, its effect at 500 µg mL^{-1} and 1000 µg mL^{-1} was statistically comparable to Teldor® when used in preventive treatment. Moreover, small activity of the extract was observed at 100 µg mL^{-1} in preventive treatment comparable to the effect of peracetic acid.

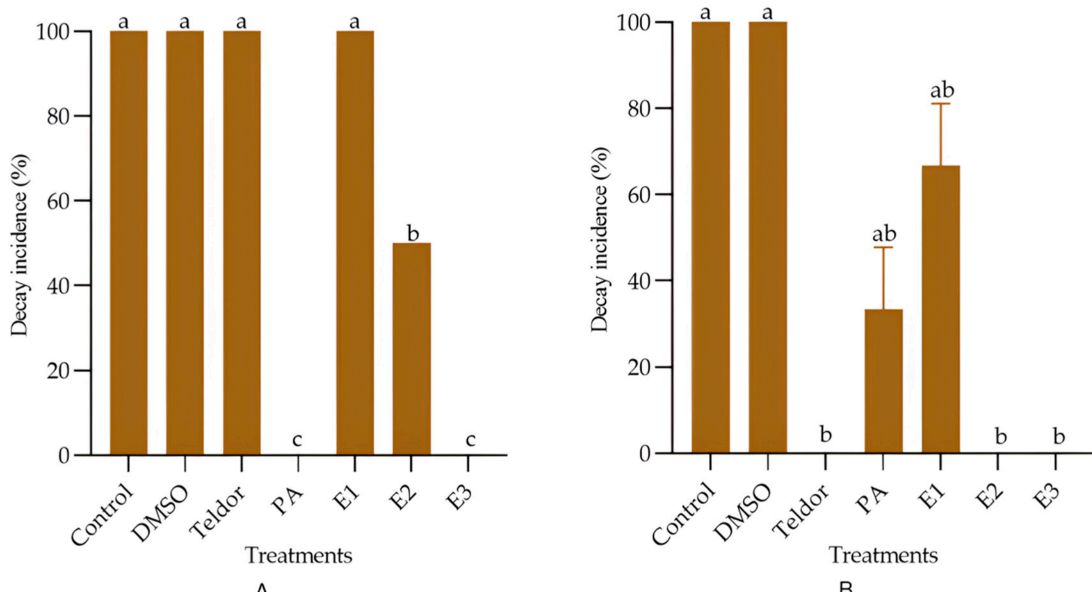

Figure 2. Effect of *Salvia discolor* dichloromethane extract on post-harvest disease caused by *B. cinerea* compared to Teldor® and peracetic acid (PA). (**A**) Curative treatment. (**B**) Preventive treatment where E1 (100 µg mL^{-1}), E2 (500 µg mL^{-1}), and E3 (1000 µg mL^{-1}) are different concentrations of the dichloromethane extract. Values followed by different letters are significantly different from each other according to Tuckey's test at $p \leq 0.05$.

3.4. Antibacterial Assays

The *S. discolor* extract was tested against two species of phytopathogenic bacteria (*C. michiganensis* subsp. *michiganensis*, and *P. carotovorum* subsp. *carotovorum*). Bacitracin and streptomycin sulfate were used as standard agents against Gram-positive bacteria [77,78]. Ampicillin and streptomycin sulfate were also used as reference antibiotics against Gram-negative bacteria [79–82]. The crude extract showed an MIC value of 500 µg mL^{-1} against *C. michiganensis* subsp. *michiganensis* and 1000 µg mL^{-1} against *P. carotovorum* subsp. *carotovorum*. Bacitracin showed an MIC value of 0.0312 µg mL^{-1} against *C. michiganensis* subsp. *michiganensis*. Streptomycin sulfate showed an MIC value of 0.25 µg mL^{-1} against both *C. michiganensis* subsp. *michiganensis* and *P. carotovorum* subsp. *carotovorum*. Ampicillin showed an MIC value of 0.0625 µg mL^{-1} against *P. carotovorum* subsp. *carotovorum*. Growth of each bacterium was observed in drug-free wells (growth control), and no growth was observed in wells containing uninoculated sterile Mueller–Hinton broth (MHB) as a blank control.

4. Discussion

The importance of safeguarding against phytopathogens has been acknowledged for centuries, dating back to the ancient use of chemical and organic pesticides [12]. Excessive pesticide application can result in the accumulation of chemical residues on crop surfaces,

promoting the development of resistant pest populations. This not only poses potential health risks to consumers but also adversely affects environmental health by disrupting ecosystems and contaminating soil and water sources [83,84]. Even though researchers are always finding and developing novel active compounds for crop protection, the process of discovery is still difficult [85], as active substances have to live up to ever-higher consumer standards and legal restrictions [86]. In the present study, the surface dichloromethane extract of *Salvia discolor* was analyzed to identify its chemical constituents and evaluate its biological activity against phytopathogenic organisms. Chemical analysis of the surface extract revealed the presence of several known terpenoids and flavonoids. Among these, diterpenoids are particularly noteworthy due to their broad spectrum of biological activities, including cytotoxic, antimicrobial, antiprotozoal, antioxidant, phytotoxic, and insecticidal effects, highlighting their importance in plant defense mechanisms against phytopathogens [22,87]. Previous research has shown that extracts and essential oils from *Salvia* and other plant species have significant effects against fungal phytopathogens [19,27,56,88]. The extract of *S. discolor* displayed the best antifungal efficacy at higher concentrations against *P. chlamydospora*, and was also active against *F. solani* with an over 90% inhibition of mycelial growth. The activity of the extract could be attributed to the presence of diterpenes, as previous studies have demonstrated the antifungal properties of these compounds [7]. These results are very promising as *P. chlamydospora* is the primary pathogen responsible for Esca disease in grapevines and the tested extract showed that 50% of growth can already be inhibited at a concentration of 71.73 $\mu g\ mL^{-1}$, highlighting its potential as an effective antifungal agent [89]. These findings are consistent with the results of Cobos et al. [90], who tested various natural antifungals against *P. chlamydospora*, showing that chitosan oligosaccharide (1000 $\mu g\ mL^{-1}$) and garlic extract both achieved the 100% inhibition of in vitro mycelial growth. Furthermore, the extract showed low effects against *A. solani*, while previous research reported moderate antifungal activity of ethanolic extracts of *S. fructicosa* against two strains of the same pathogen [91].

Botrytis cinerea poses a significant threat to crop production in various fields and greenhouse crops. The extract of *S. discolor* showed low efficacy against strains of *B. cinerea* from heavily treated crops such as *S. lycopersicum* (strain 2) and *Fragaria* × *ananassa* (strain 11), with inhibition levels lower than those achieved by the synthetic fungicide Switch®. Similar activity was observed by the crude extract of *S. somalensis* against highly treated strains of *B. cinerea* [52]. The extract of *S. discolor* was inactive against the other tested strains 1 and 4 obtained from *Lavandula angustifolia* and *Viburnum opulus*, respectively. This reduced efficacy may result from resistance developed in these crops due to frequent pesticide treatments.

The efficacy of the extract of *S. discolor* was also tested against gray mold disease in postharvest tomato fruits. At 500 $\mu g\ mL^{-1}$, the extract showed moderate efficacy in curative applications, while at 1000 $\mu g\ mL^{-1}$ no fruit decay was observed. These findings are significant, as the use of chemical treatments on postharvest fruits can leave harmful residues, making them unsafe for consumption [92].

Finally, the surface extract also exhibited moderate inhibition against the phytopathogenic bacterium *C. michiganensis* subsp. *michiganensis* (MIC value 500 $\mu g\ mL^{-1}$), which causes bacterial wilt and canker in tomatoes. This bacterium is widely considered to be the most important bacterial disease affecting tomatoes, resulting in significant commercial losses worldwide [93]. Although the MIC values were higher than those of standard antibiotics, the efficacy of the extract against this bacterium suggests its potential as a natural antibacterial agent, especially considering increasing antibiotic resistance and the prohibition of antibiotic use in agriculture in Europe. Furthermore, except for copper, no antibacterial chemicals are available for managing bacterial diseases in plants [94]. Our

results are consistent with the MIC value of 500 μg mL^{-1} shown by the aqueous methanol (1:1, *v*/*v*) leaf extract of *Ginkgo biloba* [95] and the *Silybum marianum* aqueous extract [96] against *C. michiganensis* subsp. *michiganensis.* Other plant extracts showed similar results: *Rubus*, *Anvillea* and *Pistacia* aqueous extracts had MICs equal to 3125 μg mL^{-1} [97]. In a recent study, the essential oil of *Salvia hydrangea* showed an MIC value of 500 μL mL^{-1} against the same pathogen [98]. On the other hand, the activity of the surface extract against *P. carotovorum* subsp. *carotovorum* (MIC value 1000 μg mL^{-1}) was weak compared to other results in the literature [99–102].

5. Conclusions

The current study proves that the surface extract of *Salvia discolor* has potential as a crop protectant. These results validate the first goal of assessing *S. discolor* as an alternative, sustainable substitute for traditional agrochemicals. To realize its full potential, further research on formulation strategies, synergistic blends, and applications under field conditions is needed. This would facilitate its use in integrated pest management programs and support the development of safer, environmentally friendly options for modern agriculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15061268/s1: Figure S1. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **1**; Figure S2. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **2**; Figure S3. 1H NMR (600 MHz, $CDCl_3$) spectrum of compound **3**; Figure S4. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **4**; Figure S5. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **5**; Figure S6. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **6**; Figure S7. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **7**; Figure S8. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **8**; Figure S9. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **9**; Figure S10. ^1H NMR (600 MHz, $CDCl_3$) spectrum of compound **10**; Figure S11. Inhibition of growth of *Fusarium solani* mycelium 5 days after inoculation on poisoned media; Figure S12. Inhibition of growth of *Phaeomoniella chlamydospora* mycelium 5 days after inoculation on poisoned media; Figure S13. Inhibition of growth of *Pythium dissocotum* mycelium 4 days after inoculation on poisoned media; Figure S14. Inhibition of growth of *Botrytis cinerea* (strain 11) mycelium 5 days after inoculation on poisoned media; Figure S15. Effect of treatment dose on fungal growth inhibition across different species; Information about *Salvia discolor* Kunth F.W.H.von Humboldt.

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