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Antifungal Potential of Carnosic Acid from *Salvia somalensis* against Phytopathogenic Fungi

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Abstract: The aims of the present study were (i) to characterize the dichloromethane extract of the fresh aerial parts of *S. somalensis*, cultivated in Liguria (Italy), (ii) to quantify carnosic acid production and (iii) to find an eco-friendly alternative approach to control diseases caused by phytopathogenic fungi. The phytochemical investigation yielded several known terpenoids, as well as a diterpene, 4α,9α-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one, not previously described as a plant metabolite before. The results showed a noteworthy quantity of carnosic acid (113.90 µg/mg of dried extract). The potential antifungal activity of the plant surface extract and carnosic acid against five phytopathogenic fungi (*Colletotrichum coccodes*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Rhizoctonia solani*) was considered. A complete inhibition of *C. coccodes*, *S. sclerotiorum* and *R. solani* mycelium growth was observed by carnosic acid at 500 µg/mL. High inhibition values were observed against *B. cinerea* and *F. oxysporum* compared to reference active ingredients. Four different *B. cinerea* strains exhibited a pronounced sensitivity to carnosic acid, as well as those originating from agricultural crop scenarios where a high load of active ingredient for gray mold control was historically adopted. Additionally, the formation and development of the germinative tube in *B. cinerea* were greatly slowed down.

Keywords: *Salvia somalensis*; *Salvia* spp.; carnosic acid; phytopathogenic fungi; *Botrytis cinerea*; plant disease; germinative tube; mycelium growth

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1. Introduction

Plant disease control is a tremendous challenge considering the annual losses of crops worldwide [1]. The massive use of bioactive synthetic compounds raises significant environmental concerns, resulting in the contamination of terrestrial and aquatic ecosystems, and posing risks to environmental and human health [2]. Conventional agriculture has been characterized by the emergence and proliferation of microbial resistance, excessive use of mineral fertilizers, and the absence of suitable crop rotation [3]. Many researchers are actively collaborating to explore eco-friendly alternatives for sustainable farming practices, and to promote the responsible use of agrochemicals [4]. Plant pathogens and pests cause a loss of up to 40% of the yield of economically important crops every year [5]. Among them, fungi cause more economic damage than any other group of microorganisms [6,7]. These phytopathogens includes *Colletotrichum coccodes*, a species involved in solanaceous antrachnose diseases, *Fusarium oxysporum*, responsible for vascular wilt in several growing

scenarios, *Sclerotinia sclerotiorum*, causing basal and stem rot or white mold in a wide range of hosts, *Rhizoctonia solani*, a soil-borne pathogen leading to damping-off and basal rot in many crops, and *Botrytis cinerea,* the causal agent of gray mold and one of the most important plant pathogens of worldwide interest [8–10]. The damage caused by these fungi extends to cereals, legumes, vegetables, fruit and berry crops, ornamental and other crops with a massive impact on global agriculture. *B. cinerea* is a well-known necrotrophic phytopathogen that can infect a wide range of plants, including many economically important crops, ornamental plants and fruits [11]. In the Liguria region (Italy), *B. cinerea* is considered a key pathogen affecting aromatic protected and potted crops (i.e., *Ocimum basilicum*, *Salvia* spp., *Thymus* spp. (especially during the propagation phase), horticultural crops (i.e., Tomato cv Cuore di Bue), fruit crops (i.e., grapes in the viticulture of upcountry areas) and ornamental crops (cut flowers including *Ranunculus asiaticus* (Persian buttercup), *Papaver nudicaule* (Iceland poppy), *Anemone coronaria*, *Rosa* spp. and several ornamental potted species). Gray mold caused by *B. cinerea* is a fungal disease responsible for significant losses during the storage of fruit and vegetables, particularly problematic in vineyards, affecting grapes, but it can also impact strawberries, tomatoes, lettuce, flowers and more [12]. The management of gray mold disease has historically been achieved both by frequent application of synthetic fungicides and climate monitoring and management, particularly under protected crops [13]. Synthetic fungicides against *B. cinerea* available to date can be classified into five categories according to their biochemical modes of action: fungicides affecting fungal respiration, microtubule assembly, osmoregulation, methionine biosynthesis or sterol biosynthesis [14,15]. A wide array of fungicides that belong to several target-site-specific chemical classes, such as the anilinopyrimidines (Aps; FRAC 9), sterol biosynthesis inhibitors class III and SBIs class III (FRAC 17), phenylpyrroles (PPs; FRAC 12), quinone outside inhibitors (QoIs; FRAC 11), succinate dehydrogenase inhibitors (SDHIs; FRAC 7), benzimidazoles (MBCs; FRAC 1) and dicarboximides (DCs; FRAC 2), are available against the pathogen [16,17]. However, the use of them has been restricted because of resistance associated with target site modifications and the overexpression of efflux transporters [18,19]. The increase of synthetic substances production in the last decades has highlighted the need to develop sustainable disease control strategies by reducing toxicological risks [20]. Gray mold control under organic agriculture scenarios relies particularly on agronomical tools and strategies, biocontrol agents, natural extracts and climate monitoring and management [21]. Prior to 1950, *B. cinerea* management practices were restricted to cultural methods; for example, the avoidance of highly susceptible cultivars, short-term cropping to limit inoculum build up and limited use of nitrogen fertilizer to suppress canopy development. One promising alternative for gray mold management is the use of microorganisms as biocontrol agents (BCAs). BCAs may suppress *B. cinerea* via various modes of action, including competition for nutrients and space, antibiosis, parasitism and induced host-plant resistance [22,23]. Among alternative methods of gray mold control, the use of natural compounds as plant extracts is one which can be characterized by reduced toxicity for humans and environment, selectivity, biodegradable activity and a great variety of chemical composition, with a large variety of secondary metabolites [24,25].

The genus *Salvia* (Lamiaceae) [26,27] is a rich source of bioactive diterpenoids [28–32], many of them showing antibacterial and antifungal properties, with potential as biopesticides for plant disease control. Carnosic acid is an abietane-type diterpene [33–35], increasingly recognized for its antibacterial and antifungal properties, and it has been described as one of the major known constituents in *Salvia* species [36–43]. Carnosic acid has revealed potential against common crop fungal pathogens [44]. Limited studies have explored the use of natural compounds, including carnosic acid, for crop protection and as environmentally friendly alternatives to harmful synthetic pesticides [39]. Specifically, carnosic acid has been reported to inhibit some pathogenic fungi, which are known to cause significant post-harvest losses in crops, including *B. cinerea*, *F. oxysporum*, *A. alternata*, *Aspergillus tubingensis* and *Penicillium digitatum* [38,39]. Diterpenoids showing abietane skeletal types are mainly reported in the aerial parts of Old World *Salvia* species [45], and

a recent study reported that *S. somalensis* Vatke is characterized by a relevant amount of carnosic acid [46]. *S. somalensis* [27,47] is a perennial shrub, native to Somalia, and recently introduced in the Ligurian cultivations of ornamental species for the ease of growth in the Ligurian climate, the fragrance and the color of the flowers. Generally, such species is grown in open field conditions, being better adapted for pots with a diameter higher than 16 cm. Due to the secretion of defense compounds onto the plant cuticular layer [48–52], the aims of the present study were (i) to characterize the dichloromethane extract of the fresh aerial parts of *S. somalensis* cultivated in Liguria, (ii) to quantify its carnosic acid concentration and (iii) to explore the potential of this compound as an agrochemical for the treatment of phytopathogenic fungi. For this purpose, five fungal species, i.e., *C. coccodes*, *F. oxysporum*, *S. sclerotiorum*, *B. cinerea* and *R. solani*, were considered. Taking into account the prominent evolution of *B. cinerea* resistance and lacking new antifungal compounds, a particular focus was given to *B. cinerea*, extremely affecting our region (Liguria, Italy), also considering the conidial germination and germ tube elongation assay.

2. Materials and Methods

2.1. Plant Material

The fresh aerial parts of commercial *S. somalensis* plants were obtained from the Centro Regionale di Sperimentazione ed Assistenza Agricola (CeRSAA), Albenga (SV), Italy (see cultivation technique of the species in Supplementary Materials). The plant material was identified by G.L.C. Bramley, and a voucher specimen (INTERREG III ALCOTRA, Progetto N° 074 "Sviluppo a Scopi Commerciali delle Potenzialità del Genere *Salvia* L.") was deposited at the Kew Herbarium (K).

2.2. Extraction and Isolation

2.2.1. 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).

2.2.2. General Experimental Procedures

TLC and MPLC were performed as previously described [53,54]. 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 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 µm) (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 as 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 C_{18} SymmetryPrep column, 7.8 × 300 mm ID, 7 µm particle size (Waters) was used for semi-preparative HPLC, at room temperature, flow rate 2.0 mL/min, sample injection volume 100 µL. The mobile phase was composed of A: H_2O, B: CH_3OH, at various ratios. 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). NMR data were recorded on a Bruker Avance III NMR spectrometer 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. ESIHRMS spectra were recorded on a LTQ Orbitrap XL hybrid ion trap—Orbitrap mass spectrometer (Thermo-Fisher Scientific, Waltham, MA, USA). Optical rotations were measured with a Perkin-Elmer 241 polarimeter (Perkin Elmer, Inc. Waltham, MA, USA) equipped with a sodium lamp (589 nm) and a 10 cm microcell. UV and ECD spectra were recorded in MeOH (40–400 μg/mL) on a Chirascan CD spectrometer using 1 mm path precision cells (110 QS) (Hellma Analytics, Müllheim, Germany). Data analysis was performed with Pro-Data V2.4 software.

2.2.3. Chemical Investigation of Leaf Surface Constituents

For the isolation of leaf surface constituents, fresh aerial parts of *S. somalensis* (1.5 kg) were immersed in CH_2Cl_2 for 20 s, preventing the extraction of inner cell wall components. After filtration, the extraction solvent was removed under reduced pressure. The extract (16.2 g, 1.1% *w*/*w* of fresh plant) was treated with *n*-hexane to afford an *n*-hexane-soluble (12.1 g) and an *n*-hexane-insoluble portion (3.1 g).

The *n*-hexane-soluble portion was chromatographed in aliquots of 1.0 g on a Sephadex LH-20 column (53 × 2.5 cm), using $CHCl_3/CH_3OH$ (7:3) as an eluent. The eluate fractions (20 mL each) were combined according to their TLC patterns to give five fraction groups (I–IV): group I (1.3 g) (up to 180 mL) with waxy compounds; group II (2.5 g) (from 180 to 220 mL); group III (3.6 g) (from 220 to 240 mL), and group IV (2.0 g) (from 240 to 320 mL). Each group was then separated by MPLC, and the compounds were purified by semi-preparative HPLC.

Fractionation of group I afforded 13 fractions (I_1–I_{13}). Semipreparative HPLC purification (65% CH_3OH in H_2O for 70 min, followed by 100% for 10 min) of fraction I_4 (160 mg) afforded **1** (1.5 mg, t_R 18.4 min) and **2** (1.2 mg, t_R 19.7 min). Fraction I_7 (69 mg) (HPLC method as fraction I_4) afforded **3** (1.1 mg, t_R 14.8 min). Fraction I_9 (67 mg) afforded **4** (3.0 mg, t_R 11.1 min). Fractionation of group II afforded 13 fractions (II_1–II_{13}). HPLC purification (HPLC method as fraction I_4) of fraction II_9 (320 mg) afforded **5** (4.7 mg, t_R 29.7 min), **6** (0.8 mg, t_R 30.2 min), **7** (1.1 mg, t_R 44.4 min) and **8** (0.7 mg, t_R 44.8 min). Fraction II_{10} (356 mg) was purified by semipreparative HPLC (linear gradient from 5% CH_3OH in H_2O to 100% CH_3OH in 61 min and followed by elution with 100% CH_3OH to 90 min), affording **9** (2.8 mg, t_R 61 min), **10** (1.4 mg, t_R 70.2 min), **11** (3.9 mg, t_R 74.2 min), **12** (1.3 mg, t_R 75.0 min), **13** (2.5 mg, t_R 78.2 min), **14** (7.1 mg, t_R 82.0 min) and **15** (9.3 mg, t_R 83.0 min). Fraction II_{11} (387 mg) was purified as the previous one, affording 14 (47.5 mg, t_R 82.0 min) and 15 (50.3 mg, t_R 83.0 min). Fractionation of group III afforded 13 fractions (III_1–III_{13}). Fraction III_7 (145 mg) was purified by semipreparative HPLC (65% CH_3OH in H_2O for 70 min, followed by 100% for 10 min), affording **16** (0.4 mg, t_R 27.4 min) and **17** (3.1 mg, t_R 64.2 min). Fraction III_9 (156 mg) was purified by semipreparative HPLC (linear gradient from 5% CH_3OH in H_2O to 100% CH_3OH in 61 min and followed by elution with 100% CH_3OH to 90 min), affording **12** (1.0 mg, t_R 75 min). Fraction III_{12} (347 mg) was purified by semipreparative HPLC (60% CH_3OH in H_2O for 30 min, and followed by 100% for 20 min), affording **18** (10.5 mg, t_R 12.0 min) and **19** (20.1 mg, t_R 16.1 min). Fraction III_{13} (451 mg) was purified by semipreparative HPLC (linear gradient from 5% CH_3OH in H_2O to 100% CH_3OH in 61 min and followed by elution with 100% CH_3OH to 90 min), affording **14** (20.5 mg, t_R 82.0 min) and **15** (13.3 mg, t_R 83.0 min). Fractionation of group IV afforded 15 fractions (IV_1–IV_{15}). HPLC purification (65% CH_3OH in H_2O for 70 min, followed by 100% for 10 min) of fraction IV_8 (146 mg) afforded **17** (1.0 mg, t_R 64.0 min), **20** (1.5 mg, t_R 16.2 min), **21** (0.3 mg, t_R 16.5 min), and **22** (0.4 mg, t_R 24.5 min). Fraction IV_9 (234 mg) (HPLC method as fraction IV_8) afforded **23** (1.7 mg, t_R 5.8 min), **24** (1.3 mg, t_R 8.0 min) and **25** (1.1 mg, t_R 25.3 min). Fraction IV_{10} (148 mg) (HPLC method as fraction IV_8) afforded **26** (1.4 mg, t_R 11.5 min), **27** (0.4 mg, t_R 16.5 min) and **28** (0.4 mg, t_R 19.5 min). Fraction IV_{13} (143 mg) (HPLC method as fraction IV_8) afforded **29** (2.9 mg, t_R 14.9 min) and **30** (10.2 mg, t_R 18.2 min). Fraction IV_{15} (126 mg) (HPLC method as fraction IV_8) afforded **31** (1.3 mg, t_R 6.7 min), **32** (0.8 mg, t_R 9.3 min) and **33** (1.4 mg, t_R 23.2 min).

The *n*-hexane-insoluble portion chromatographed in aliquots of 1.0 g by Si gel MPLC eluting with *n*-hexane/ethyl acetate at concentrations varying from 91:9 to 76:24 to obtain 12 fractions (V_1–V_{12}). HPLC purification of fraction V_9 (533 mg) was purified by semipreparative HPLC (60% CH_3OH in H_2O for 30 min, and followed by 100% for 20 min), afforded **19** (197.6 mg, t_R 16.1 min). Fraction V_{10} (474 mg) was purified by semipreparative HPLC with the same method as the previous fraction, affording **18** (57.3 mg, t_R 12.0 min) and **19** (142.7 mg, t_R 16.1 min). Fraction V_{11} (498 mg) (HPLC method as fraction V_{10}) afforded **18** (153.6 mg, t_R 12.0 min). Fraction V_{12} (146 mg) (HPLC method as fraction V10) afforded **13** (28.4 mg, t_R 16.2 min) and **18** (34.5 mg, t_R 12.2 min).

4α,9α-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one (**25**): ^{1}H NMR (500 MHz, CD_3OD): 6.83 (s, 1H), 2.93 (septet, *J* = 6.9, 6.9, 1.1 Hz, 1H), 2.82 (d, *J* = 18.5 Hz, 1H), 2.47 (d, *J* = 18.5 Hz, 1H), 2.12 (td, *J* = 12.9, 12.8, 7.0 Hz, 1H), 1.96–1.94 (m, 2H), 1.82 (m, 1H), 1.78 (m, 1H), 1.66 (td, *J* = 13.7, 13.7, 4.1 Hz, 1H), 1.49 (m 1H), 1.46 (m 1H), 1.32 (m, 1H), 1.24 (m, 1H), 1.17 (s, 3H), 1.12 (m, 1H), 1.10 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 0.96 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): 182.2, 143.9, 142.0, 140.2, 134.8, 83.3, 80.9, 45.9, 41.6, 40.9, 37.5, 35.4, 34.3, 31.1, 26.4, 23.3, 21.1, 20.5, 19.2, 18.7.

2.2.4. Computational Methods

Conformational analysis was performed with Schrödinger MacroModel 11.0 (Schrödinger, LLC, New York, NY, USA) employing the OPLS2005 force field in H_2O for geometrical optimization in two steps. In the first step, a global minimum was searched using 30,000 steps. In the second step, the global minimum was used as input for a conformational search over 10,000 steps to choose five to seven conformers for ab initio calculations. These were subjected to geometrical optimization and energy calculation applying DFT with the Coulomb-attenuating method, Becke's nonlocal three-parameter exchange and correlation functional and the Lee–Yang–Parr correlation functional level (CAM-B3LYP), together with the B3LYP/6-31+G(d,p) basis set, the SCRF method and the CPMC model for solvation (in MeOH) with the Gaussian 09 program package [55]. Excitation energy (denoted by wavelength in nm), rotator strength (R_{str}), dipole velocity (R_{vel}) and dipole length (R_{len}) were calculated in MeOH by TD-DFT/CAM-B3LYP/6-31+G(d,p). ECD curves were obtained based on rotator strengths with a half-band of 0.25 eV (if not otherwise noted) using SpecDis v1.71 [56]

2.2.5. HPLC Quantitative Analysis

The quantification of carnosic acid (**19**) in the leaf surface extract was carried out through µHPLC 1100 (Agilent Agilent Technologies, Palo Alto, CA, USA) equipped with Symmetry C18 column (1 mm × 150 mm, 3.5 µm particle size, Waters S.p.A., Sesto San Giovanni, Italy), at room temperature, and gradient elution with a binary solution (eluent A: H_2O/HCOOH 0.1% *v*/*v*, eluent B: CH_3CN/HCOOH 0.05% *v*/*v*). The analysis started with 15% of B, then was increased to 100% over 35 min, then kept at 100% for 5 min and finally re-equilibrated to 15% B and held for 15 min. The flow rate was 30 µL/min, and the injection volume was 8 µL. UV detection was monitored at 280 nm. The calibration curve for **19** was obtained at a concentration range from 1 µg/mL to 6 µg/mL. The linearity of the instrumental response in the analyzed concentration range was confirmed, as inferred by the following fitting curve parameters: y = 8.1093 x + 2.6092; R^2 = 0.9996. The limit of detection (LOD) and limit of quantification (LOQ) were determined by serial dilution of **19** until the signal-to-noise (S/N) ratio was 3 for LOD and 10 for LOQ. The obtained values of LOD and LOQ were 1 pmol/µL and 3.6 pmol/µL, respectively.

2.3. Antifungal Assays

2.3.1. Fungi Strains

Strains of *C. coccodes*, *F. oxysporum*, *S. sclerotiorum*, *R. solani* and *B. cinerea* have been isolated at CeRSAA (Albenga (SV), Italy) from symptomatic plants of *Solanum lycopersicum*, *Cyclamen persicum*, *Lactuca sativa*, *Ocimum basilicum* and *Fragraria x ananassa*, respectively,

and used in a first antifungal activity assay. The same *B. cinerea* strain was used in the conidial germination and germ tube elongation assay. Furthermore, four *B. cinerea* strains isolated from symptomatic plants of *Solanum lycopersicum* (strain 2), *Fragraria x ananassa* (strain 3), *Ocimum basilicum* (strain 7) and *Lavandula angustifolia* (strain 12) were tested together in a second antifungal activity assay. For the isolation portions, infected plant material was washed with water and cut into small pieces to be transferred into Petri dishes, containing approximately 30 mL of Potato Dextrose Agar (VWR Life Science, Milano, Italy) supplemented with 5 mg/L streptomycin sulphate (VWR Life Science, Milan, Italy) to prevent bacterial contamination. The Petri dishes were incubated for 7 days at 27 ± 2 °C in the dark. The plates were examined day-by-day to observe mycelium growth. Pure colonies of fungi strains were then isolated and incubated for 7 days at 27 ± 2 °C, in the dark. The fungi were finally identified using morphological and microscopic analysis and characterized by molecular sequencing of the ITS region, following the method reported by White et al. [57]. The pathogenicity of the strains was confirmed in vivo (Koch's Postulates confirmation) [58,59].

2.3.2. Antifungal Activity Assay

Firstly, the inhibitory effect of *S. somalensis* surface dichloromethane extract and carnosic acid on mycelium growth of *C. coccodes, F. oxysporum, S. sclerotiorum, R. solani* and *B. cinerea* was tested. The bottles, containing culture medium with 39 g of Potato Dextrose Agar (Merck, Milan, Italy) and 1 L of distilled water, were autoclaved for 15 min at 121 °C and then cooled to 50 °C. *S. somalensis* crude surface exudate, carnosic acid and the four synthetic fungicides were added separately. Extract (solubilized in DMSO 1% *v*/*v*) was tested at 500 and 1000 µg/mL. Carnosic acid (solubilized in DMSO 1% *v*/*v*) was tested at the following concentrations: 5, 10, 50, 250, 500, 750, 1000 µg/mL. The fungicides concentrations are those recommended by manufacturers: Bavistin® FL (BASF, Rome, Italy) (carbendazim 41%), carbendazim 500 µg/mL; Rovral® FL (BASF, Rome, Italy) (iprodionee 25%), iprodionee 675 µg/mL; Ortiva® (Syngenta, Milan, Italy) (azoxystrobin 23%), azoxystrobin 250 µg/mL; Switch® (Syngenta, Milan, Italy) (cyprodinil 37% + fludioxonil 25%), cyprodinil 375 and fludioxonil 250 µg/mL, respectively. The obtained media were then supplemented with streptomycin sulphate (5 µg/mL) and solidified in 90 mm diameter Petri dishes. Mycelium agar plugs (<2 × 2 mm) were placed on the medium, in sterile conditions, directly in the center of the Petri dishes [60], which were incubated in the dark at 25 °C. Then, carnosic acid was tested on mycelium growth of four *B. cinerea* strains, following the same conditions previously described. The concentration of the employed fungicide is that recommended by the manufacturer: Switch® (Syngenta, Milan, Italy) (cyprodinil 37% + fludioxonil 25%), at 375 and 250 µg/mL, which is specific for *B. cinerea* [60]. Each test included the evaluation of growth radius on poisoned medium with DMSO 1% *v*/*v* and not poisoned medium (control). Three replicates were made for each concentration. The radial mycelial growth of the pathogens was assessed by measuring the colony radius in millimeters 5 days after the inoculation and the percentage of inhibition exerted by the test substances compared to the untreated control was determined. The experiments were repeated three times, and all three tests gave comparable results.

2.3.3. *B. cinerea* Conidial Germination and Germ Tube Elongation Assay

The effect of carnosic acid upon the germ tube elongation of conidia of *B. cinerea* were evaluated according to Leroux et al. [61]. An amount of 0.3 mL of a conidial suspension (2×10^5 conidia mL^{-1}) was spread on the surface of an agar medium containing 10 g glucose, 2 g K_2HPO_4, 2 g KH_2PO_4, and 12.5 g agar for 1 L [62]. The bottles with the medium were autoclaved for 15 min at 121 °C and then cooled to 50 °C. Carnosic acid (solubilized in DMSO 1% *v*/*v*) and the four synthetic fungicides were added separately. Carnosic acid was tested at the following concentrations: 5, 10, 50, 250, 500, 750, 1000 µg/mL. The concentrations at which fungicides have been used are those recommended by manufacturers. The obtained media were then added with streptomycin sulphate (5 µg/mL) and solidified

in 90 mm diameter Petri dishes. Each test was repeated three times. The phenotypic characterization was completed after an incubation of 24 h at about 21 °C. Then, the number of germinated conidia was counted, and this count was repeated after 48 h. The percentage of conidia germinated after 24 and 48 h on the substrates poisoned with the test substances compared to the untreated control was then determined, using a microscope Nikon Eclipse 55 (zoom 10×). After 48 h, mycelium agar plugs of each replication were transferred on PDA to verify the viability of the conidia. Each test included the evaluation of the conidial germination on medium poisoned with DMSO 1% *v*/*v* and not poisoned (control).

2.3.4. Statistical Analysis

The effect of treatments was assessed using the analysis of variance (ANOVA), followed by Tukey HSD test mean separation at $p \leq 0.05$. Statistics were performed by using the "Statistica" software package, version 8.0, Statsoft Inc. Tulsa, OK, USA [63].

3. Results

3.1. Chemical Investigation of Leaf Surface Constituents

The dichloromethane extract of the plant surface of *S. somalensis* was analyzed by the chromatographic method, and afforded several known compounds (Table S1, Supplementary Materials), identified by spectroscopic NMR analysis, including DEPT, TOCSY, HSQC and HMBC experiments, ESIHRMS and ECD calculations, and comparison with literature data. The more lipophilic portion afforded several sesquiterpenoids named (-)-7-*epi*-isojunenol (**1**) [64], (+)-junenol (**2**) [65], (+)-*ent*-epicubenol (**3**) [66], teucladiol (**4**) [67], 4β,6β-dihydroxy-1α,5α(H)-guai-9-ene (**11**) [68], β-chaenocephalol (**23**) [69], 1β,6β-dihydroxy-4(14)-eudesmene (**24**) [70,71] and 3,11-dihydroxy-3,7,11-trimethyldodeca-1,6,9-triene (**31**) [72]. Triterpenoids, betulin (**6**) [73], uvaol (**7**) [74], betulinic acid (**8**) [73], oleanolic acid (**14**) [75], ursolic acid (**15**) [75] and one flavonoid, 5-hydroxy-7,4′-dimethoxyflavone (apigenin-7,4′-dimethyl ether) (**12**) [76], were also present. Diterpenoid compounds, 7,13-*E*-labdadien-15-ol (**17**) [77], the abietanes [33] pisiferol (**5**) [78,79], rosmanol (**9**) [80,81], rosmadial (**10**) [82], 12-methylcarnosic acid (**13**) [83,84], 12-*O*-methyl-acetylcarnosate (12-methoxy-11-hydroxy-8,11,13-abietatrien-2-oic acid methylester) (**16**) [85], 14-hydroxy-7-*O*-methylrosmanol (**27**) [86], 12-methylcarnosol (**28**) [87], galdosol (**26**) [88,89], carnosol (**18**) [84], carnosic acid (**19**) [84], 20-deoxocarnosol (**20**) [90], 11-hydroxy-12-methoxyabieta-8,11,13-triene (**21**) [91], ferruginol (**22**) [92], 11-hydroxysugiol (**32**) [93], formosanoxide (**33**) [94], and the icetexane brussonol (**29**) [91,95], demethylsalvicanol (**30**) [91] and 4α,9α-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one (**25**) [95] were isolated.

Spectroscopic data of compound **25** (Figures 1 and S1–S6, Supplementary Materials) allowed it to be identified as an icetexane compound, previously described by Majetich and Zou [95] as a synthetic product obtained by treatment of an *o*-quinone intermediate of synthesis of (+)-brussonol with zinc chloride. To the best of our knowledge, this compound has never been isolated from a plant source before. In the study by Majetich and Zou [95], NMR data were not reported, and the absolute configuration of **25** was not established. To better describe compound **25**, its absolute configuration was studied by electronic circular dichroism (ECD). The experimental ECD spectrum of **25** (Figure S7, Supplementary Materials) showed a pronounced positive Cotton effect (CE) at 309 nm, strong negative CEs at 214 and 353 nm, and a weak negative CE at 260 nm. The ab initio calculated ECD spectrum of **25** showed corresponding CEs for all four observed CEs. Thus, the absolute configuration of **25** was assigned as (5*S*,5*R*,10*S*)-4α,9α-epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one.

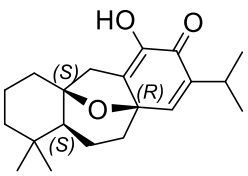

Figure 1. Structure of compound **25**.

3.2. HPLC Quantitative Analysis

The amount of carnosic acid (**18**) in the surface dichloromethane extract of *S. somalensis* was determined by HPLC-UV analysis (Figure S8, Supplementary Materials). The results showed that the content of **18** was 113.90 µg/mg of dried extract.

3.3. Antifungal Screening

S. somalensis crude surface exudate and carnosic acid were tested in vitro for antifungal activity against *C. coccodes*, *F. oxysporum*, *S. sclerotiorum*, *B. cinerea* and *R. solani* (Figure S9, Supplementary Materials). The antifungal activity was evaluated by the measurement of mycelial growth inhibition. A representative figure showing inhibition of growth of fungal mycelium after inoculation on poisoned medium with carnosic acid is reported in the Supplementary Materials (Figure S10). The results shown in Table 1 indicate that the *S. somalensis* exudate and carnosic acid affected mycelial growth of the five phytopathogens. The percentage of growth inhibition was determined by measuring the mycelial growth in the absence and presence of different concentrations of tested compounds. The activity was also compared with that of four conventional chemical fungicides. *S. somalensis* crude surface exudate showed a high percent of inhibition against all the tested fungi, ranging from 40% (at 500 µg/mL) to 85% (at 1000 µg/mL) with values comparable to the reference products used. Remarkably complete inhibition of mycelium growth was achieved by carnosic acid against *C. coccodes*, *S. sclerotiorum* and *Rhizoctonia solani* at 500 µg/mL as well as the reference product Switch® (cyprodinil 375 µg/mL and fludioxonil 250 µg/mL). Moreover, significant inhibition for *C. coccodes* and *S. sclerotiorum* was detected also at 50 µg/mL, similar to Bavistin® FL (carbendazim 500 µg/mL) and for *C. coccodes* at 5 µg/mL similar to Rovral® FL (iprodionee 675 µg/mL). Carnosic acid at 250 µg/mL gave results comparable to Ortiva® (azoxystrobin 250 µg/mL) and Rovral® FL (iprodionee 675 µg/mL) treatment when tested on *F. oxysporum*. High mycelium growth inhibition values were obtained with carnosic acid at 250 µg/mL against *S. sclerotiorum* compared to Bavistin® FL (carbendazim 500 µg/mL) treatment, while complete inhibition was observed at 500 µg/mL. Similar results were obtained against *B. cinerea* with carnosic acid at 250 µg/mL compared to the reference products Switch® (cyprodinil 375 µg/mL and fludioxonil 250 µg/mL) and Rovral® FL (iprodionee 675 µg/mL). In summary, from a statistical point of view, the activity of carnosic acid at 500 µg/mL against *C. coccodes* was comparable with cyprodinil and fludioxonil (375 µg/mL + 250 µg/mL), the activity of carnosic acid at 750 µg/mL against *F. oxysporum* was comparable with iprodione (675 µg/mL) and the activity of carnosic acid at 1000 µg/mL against *S. sclerotiorum* was comparable with carbendazim (500 µg/mL) and cyprodinil and fludioxonil (375 µg/mL + 250 µg/mL). On the other hand, the activity of carnosic acid at 500 µg/mL against *R. solani* was statistically comparable with iprodione (675 µg/mL), carbendazim (500 µg/mL) and cyprodinil and fludioxonil (375 µg/mL + 250 µg/mL). The activity of carnosic acid at the tested concentrations against *B. cinerea* was never comparable from a statistical point of view to the activity of synthetic fungicides. However, it must be noted that in the case of carbendazim and azoxystrobin, no efficacy against *B. cinerea* was observed, showing a possible evolution of resistance in the strain tested. Then, in vitro experiments were carried out to assess carnosic acid's efficacy against four strains of *B. cinerea* sourced from different diseased crops. These crops exhibit diversity in permissible treatments for *Botrytis*, encompassing variations in both the active ingredients utilized and the frequency of application throughout the cultivation season. The percentage of growth inhibition was determined by measuring the mycelial growth in the absence and presence of different concentrations of tested compounds. The results shown in Table 2 indicate that carnosic acid affected at different levels the mycelial growth of the four strains of *B. cinerea*. Representative figures showing inhibition of growth of *B. cinerea* mycelium after inoculation on poisoned medium with carnosic acid are reported in the Supplementary Materials (Figures S10 and S11). Carnosic acid showed a dose-response effect with a level inhibition of growth greater than 50% starting from the dose of 250 µg/mL and reaching 80% inhibition at 1000 µg/mL. This is probably due to

strains coming from crops with a high anti-botrytic defense load and a greater possibility of developing resistance phenomena (*S. lycopersicum*, strain 2 and *F. x ananassa*, strain 3). In case of strain 2, the inhibition values at 750 and 1000 µg /mL were statistically comparable to each other, while for strain 3 the percentage of inhibition at 500 µg /mL was statistically comparable with both the inhibition values at 750 and 1000 µg /mL. The treatment of strain 2 and strain 3 with the synthetic product showed an inhibition of 100% in the growth of the mycelium, not statistically comparable with the action of carnosic acid. On the other hand, strains isolated from crops that are treated infrequently, either because of legal restrictions (*O. basilicum*, strain 7) or because it is not economically viable (*L. angustifolia* strain 12), showed a high sensitivity to carnosic acid even at very low doses (5 µg/mL), resulting in 100% growth inhibition and fully comparable with the formulated product.

Table 1. Percentage of mycelium's growth inhibition of five phytopathogenic fungi after the treatment with different concentrations of carnosic acid (**18**) and *S. somalensis* crude extract by comparison with four conventional fungicides.

Treatment	Concentration	*C. coccodes*	*F. oxysporum*	*S. sclerotiorum*	*B. cinerea*	*R. solani*
Carnosic acid	5 µg/mL	42.9 ± 10.6^{c}	21.7 ± 5.8^{bc}	0.0 ± 0.0^{a}	16.7 ± 5.8^{bc}	0.0 ± 0.0^{a}
Carnosic acid	10 µg/mL	54.6 ± 5.2^{de}	35.0 ± 10.0^{c}	3.3 ± 5.8^{a}	23.3 ± 5.8^{bcd}	0.0 ± 0.0^{a}
Carnosic acid	50 µg/mL	88.6 ± 4.2^{g}	65.0 ± 8.7^{e}	87.8 ± 3.8^{cd}	46.7 ± 3.3^{cdef}	25.6 ± 10.7^{b}
Carnosic acid	250 µg/mL	93.2 ± 0.3^{h}	81.7 ± 2.9^{fg}	94.4 ± 1.9^{d}	75.6 ± 8.4^{efgh}	93.3 ± 3.3^{d}
Carnosic acid	500 µg/mL	100 ± 0.0^{i}	81.7 ± 2.9^{fg}	97.8 ± 3.8^{de}	81.1 ± 1.9^{fgh}	100 ± 0.0^{e}
Carnosic acid	750 µg/mL	100 ± 0.0^{i}	86.7 ± 2.9^{g}	97.8 ± 1.9^{de}	81.1 ± 5.1^{gh}	97.8 ± 3.8^{e}
Carnosic acid	1000 µg/mL	100 ± 0.0^{i}	86.7 ± 5.8^{g}	98.9 ± 1.9^{e}	86.7 ± 0.0^{gh}	97.8 ± 3.8^{e}
Crude exudate	500 µg/mL	54.2 ± 5.9^{de}	44.0 ± 2.0^{de}	65.0 ± 1.7^{b}	45.6 ± 3.3^{cdef}	40.1 ± 3.8^{bc}
Crude exudate	1000 µg/mL	82.5 ± 0.9^{f}	80.7 ± 0.2^{fg}	84.5 ± 0.7^{cd}	67.0 ± 3.3^{def}	70.6 ± 3.7^{c}
Azoxystrobin	250 µg/mL	77.4 ± 3.5^{f}	80.0 ± 0.0^{fg}	96.7 ± 5.8^{de}	0.0 ± 0.0^{a}	31.1 ± 3.8^{b}
Cyprodinil + Fludioxinil	375 + 250 µg/mL	100 ± 0.0^{i}	96.7 ± 2.9^{h}	100 ± 0.0^{e}	96.7 ± 3.3^{h}	100 ± 0.0^{e}
Iprodione	675 µg/mL	54.8 ± 6.9^{de}	85.0 ± 0.0^{g}	97.8 ± 1.9^{de}	95.6 ± 3.8^{h}	100 ± 0.0^{e}
Carbendazim	500 µg/mL	86.4 ± 0.6^{ga}	100 ± 0.0^{i}	100 ± 0.0^{e}	0.0 ± 0.0^{a}	100 ± 0.0^{e}
DMSO	10 µL/mL	4.2 ± 3.9^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	4.4 ± 3.8^{ab}	0.0 ± 0.0^{a}
Control	-	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

Percentage of inhibition is correlated with standard deviation. Different letters indicate significant difference among treatments ($p \leq 0.05$, Tukey HSD test). Control: not treated plates.

Table 2. Percentage of mycelium's growth inhibition of four strains of *B.cinerea* after the treatment with different concentrations of carnosic acid (**18**) by comparison with the formulated product Switch® (Cyprodinil 375 µg/mL and Fludioxonil 250 µg/mL).

Treatment	Concentration	*B. cinerea* (Strain 2)	*B. cinerea* (Strain 3)	*B. cinerea* (Strain 7)	*B. cinerea* (Strain 12)
Carnosic acid	5 µg/mL	18.5 ± 0.6^{fg}	0.0 ± 0.0^{ef}	100 ± 0.0^{a}	100 ± 0.0^{a}
Carnosic acid	10 µg/mL	22.9 ± 3.6^{f}	10.0 ± 0.7^{e}	100 ± 0.0^{a}	100 ± 0.0^{a}
Carnosic acid	50 µg/mL	35.4 ± 3.6^{e}	43.8 ± 8.7^{d}	100 ± 0.0^{a}	100 ± 0.0^{a}
Carnosic acid	250 µg/mL	52.1 ± 3.6^{d}	51.8 ± 7.4^{cd}	100 ± 0.0^{a}	100 ± 0.0^{a}
Carnosic acid	500 µg/mL	64.6 ± 7.2^{c}	56.4 ± 10.3^{bc}	100 ± 0.0^{a}	100 ± 0.0^{a}
Carnosic acid	750 µg/mL	79.2 ± 3.6^{b}	75.9 ± 3.7^{bc}	100 ± 0.0^{a}	100 ± 0.0^{a}
Carnosic acid	1000 µg/mL	83.3 ± 3.6^{b}	78.1 ± 7.5^{bc}	100 ± 0.0^{a}	100 ± 0.0^{a}
DMSO	10 µL/mL	0.0 ± 0.0^{g}	0.0 ± 0.0^{f}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
Cyprodinil + Fludioxinil	375 + 250 µg/mL	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Control	-	0.0 ± 0.0^{g}	0.0 ± 0.0^{ef}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}

Percentage of inhibition is correlated with standard deviation. Different letters indicate significant difference among treatments ($p \leq 0.05$, Tukey HSD test). Control: not treated plates.

3.4. B. cinerea Conidial Germination and Germ Tube Elongation Assay

To better understand the mode of action of carnosic acid, in vitro tests have been set up to define the viability of *B. cinerea* conidia grown in poisoned medium [61]. Measurements taken 24 and 48 h after inoculation showed that carnosic acid effectively inhibits

germination and tube formation at 1000 µg/mL as well as the reference product Switch® at the dose recommended by the manufacturer (cyprodinil 375 µg/mL and fludioxonil 250 µg/mL) (Table 3). At 24 h, many replicates showed no conidial development at 1000 and 750 µg/mL with an effect statistically comparable with Switch®. On the other hand, the results observed after 48 h showed an onset of germination in carnosic acid plates at a concentration of 1000 µg/mL and, as expected, a total inhibition by the product Switch® which has a recognized fungicidal activity. Nevertheless, the shape and development of the germ tubes with carnosic acid have maintained the characteristics indicated above, not comparable to any other plate containing the fungicides used as the control. To assess the conidia viability, agar plugs with conidial suspension were transferred on a solid non-selective substrate (PDA) after 48 h. The resulting mycelium growth from the agar plugs poisoned with carnosic acid, especially at high concentrations, was in all cases very slow. Moreover, several replicates showed no conidial development at 1000 and 750 µg/mL also after 48 h.

Table 3. Effect of carnosic acid (**18**) and reference fungicides on the *B. cinerea* conidial germination after 24 h and 48 h.

Treatment	Concentration	24 h	48 h
Carnosic acid	5 µg/mL	90.2 ± 6.6 ^{de}	94.4 ± 6.9 ^{e}
Carnosic acid	10 µg/mL	82.5 ± 7.9 ^{de}	89.0 ± 15.5 ^{de}
Carnosic acid	50 µg/mL	82.4 ± 11.2 ^{cde}	80.8 ± 12.8 ^{de}
Carnosic acid	250 µg/mL	60.2 ± 20.2 ^{bcd}	74.7 ± 5.7 ^{de}
Carnosic acid	500 µg/mL	48.4 ± 24.3 ^{bc}	64.1 ± 10.7 ^{cd}
Carnosic acid	750 µg/mL	6.2 ± 6.3 ^{a}	30.1 ± 24.6 ^{b}
Carnosic acid	1000 µg/mL	0.1 ± 0.2 ^{a}	17.3 ± 10.1 ^{ab}
Carbendazim	500 µg/mL	99.9 ± 0.2 ^{e}	100 ± 0.0 ^{e}
Iprodionee	675 µg/mL	47.3 ± 2.9 ^{b}	39.6 ± 2.6 ^{bc}
Azoxystrobin	250 µg/mL	63.3 ± 21.2 ^{bcd}	89.6 ± 4.3 ^{de}
Cyprodinil + Fludioxinil	375 + 250 µg/mL	0.1 ± 0.2 ^{a}	0.4 ± 0.8 ^{a}
DMSO	10 µL/mL	98.2 ± 3.1 ^{e}	100 ± 0.0 ^{e}
Control	-	98.2 ± 3.2 ^{e}	100 ± 0.0 ^{e}

Results are expressed as the percentage of mean germination of the three repeated tests. Percentage of inhibition is correlated with standard deviation. Different letters indicate significant difference among treatments ($p \leq 0.05$, Tukey HSD test). Control: not treated plates.

4. Discussion

New compounds to replace those that have become unavailable due to toxicity or resistance in target pathogens are urgently needed [96–98]. Directive 2009/128/EC [99] about the sustainable use of plant protection products contained several measures to reduce the risks and impacts of plant protection products on human health and the environment by promoting the use of Integrated Pest Management (IPM) and alternative approaches or technologies, including non-chemical alternatives to plant protection products. Furthermore [40], Regulation (EC) No 1107/2009 has set more stringent requirements for the approval of active substances. As a result, there are many regulatory restrictions on the use of fungicides. Natural products are recognized as valuable resources in the search for novel antifungal agents [100–102]. In the present study, the dichloromethane surface extract *of S. somalensis* cultivated in Liguria (Italy) was characterized and the content of carnosic acid has been determined. The surface extract showed the presence of several terpenoids, including constituents of the essential oil and non-volatile terpenoids, and flavonoids, characteristic of trichome secretion products. These compounds have a pivotal role in plant defense, and often show antimicrobial and allelopathic activity [51,103]. Abietane and icetexane diterpenoids were isolated. Abietanes and rearranged abietane skeletons are common in *Salvia* species [29,32]. The icetexanes belonged to the structural class 2 (barbatusol family) [104,105], and have been described as biogenetically related to abietane diterpenoids. As reported for other *Salvia* spp., a significant amount of carnosic

acid (113.90 µg/mg of dry extract) was detected in *S. somalensis*. These data are consistent with those reported in the literature [40,43,106–108]. This hinted at the potential of utilizing *S. somalensis* as a viable source for extracting this bioactive compound, prompting further exploration for its formulation through new studies. Carnosic acid has been recognized as a potent antimicrobial constituent of several *Salvia* species [37]. Our results demonstrated the ability of carnosic acid to completely inhibit the mycelium growth of *C. coccodes*, *S. sclerotiorum* and *R. solani* already at 500 µg/mL. Moreover, 80% of inhibition was also observed against *F. oxysporum* and *B. cinerea* when tested at the same concentration. A very strong antifungal activity was observed against all the tested fungi already at 250 µg/mL. Relevant inhibition values were obtained at the lowest tested concentration (5 µg/mL) against *C. coccodes*, and to a lesser extent against *F. oxysporum* and *B. cinerea.* In fact, the frequency of resistance to carbendazim to *B. cinerea* due to mutations has reached high values [109]. *S. somalensis* crude surface extract showed good inhibition values, comparable to the classical chemical fungicides (iprodione and carbendazim). These results emphasize the potential of using natural compounds or extracts as fungicide and their crucial role in crop protection [110]. The effectiveness of the leaf surface extract of *S. somalensis* could be associated with a potential synergy between the constituents. Several studies have reported extracts from natural sources which exhibit antifungal activity against *B. cinerea* [111,112]. As some compounds create a membrane instability producing a permeability alteration of *B. cinerea* cytoplasmic membrane [113], while others completely inhibit conidial germination, to better investigate the mechanism of action, the efficacy of carnosic acid on *B. cinerea* conidial germination and germ tube elongation was assessed. Carnosic acid effectively inhibited germ tube formation at 1000 µg/mL after 24 h. Although the onset of germination has been observed at all other carnosic acid concentrations, the effect was comparable to the controls. Germ tube formation and development were greatly slowed down, confirming the great antifungal potential of carnosic acid. The reduction in the development of the germ tubes suggests a possible interference of carnosic acid in protein synthesis (as in the case of cyprodinil) or in the development and function of membranes (as in the case of carbamates and some plant extracts) which can usually lead to a decrease in the development of the mycelium [114]. In the Liguria region (Italy), *B. cinerea* stands out as a formidable threat due to its deleterious effects on a wide array of crops that form the backbone of agricultural and horticultural activities. This fungal pathogen's presence is particularly felt in the cultivation of aromatic plants. Beyond them, *B. cinerea*'s reach extends to embrace horticultural staples, jeopardizing harvests and livelihoods alike. Our intent was to conduct a thorough investigation into four strains of *B. cinerea* isolated from symptomatic plants. In vitro experiments revealed that significant activity was displayed at minimal concentrations (5 µg/mL), leading to complete growth inhibition, comparable to the conventional product Switch®. Carnosic acid efficacy was particularly pronounced in treating strains isolated from *O. basilicum* and *L. angustifolia*, rarely tested.

Currently, the control of gray mold is heavily reliant on single-site fungicides [115]. However, because of their interactions with defined molecular targets, with a highly specific mode of action, they are at high risk of developing resistance due to specific mutations [18]. In this sense, being able to have new possible sources of active molecules against *B. cinerea* is of primary importance because it would allow the implementation of successful strategies to delay the evolution of resistance in pathogen populations. Given these possible outcomes and the good amount of carnosic acid, *S. somalensis* could be considered a new source of bioactive compounds useful in organic farming, with low environmental impact. Furthermore, these results hold good promise for the Ligurian region, where *B. cinerea*'s impact on various crops is keenly felt.

5. Conclusions

The phytochemical investigation of *S. somalensis* surface exudate afforded a compound (**25**) that has never been isolated from a plant source before, as well as several known compounds. A remarkable quantity of carnosic acid (113.90 µg/mg of dried extract) has

been detected, emphasizing the potential of *S. somalensis* as a new source of bioactive compounds. This study also proposed the potential use of the plant surface exudate and carnosic acid as a valid eco-friendly strategy to control plant diseases. The dichloromethane extract of *S. somalensis* and carnosic acid exhibited notable efficacy against the tested pathogens giving interesting results in the antifungal screening. Four *B. cinerea* strains were identified as exhibiting a strong sensitivity to carnosic acid. These strains were isolated from agricultural crops, where they were found to have a high load of the active ingredient for gray mold control. Moreover, the results showed that the formation and development of the germinative tube in *B. cinerea* were greatly slowed down, highlighting the potential interesting use of new active ingredients to be used in organic farming. Further research is warranted to investigate the potential mechanisms of action in more complex agricultural scenarios, where the selection of naturally resistant individuals and their competition with the wild-type population may occur as a result of the intensive use of products on a large area.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14071444/s1, Figure S1. 1H NMR (500 MHz, CD_3OD) spectrum of compound **25**; Figure S2. HSQC (500 MHz, CD_3OD) spectrum of compound **25**; Figure S3. HMBC (500 MHz, CD_3OD) spectrum of compound **25**; Figure S4. COSY (500 MHz, CD_3OD) spectrum of compound **25**; Figure S5. NOESY (500 MHz, CD_3OD) spectrum of compound **25**; Figure S6. ESIMS spectrum of compound **25**; Figure S7. ECD spectra of compound **25**; Figure S8. Representative chromatograms of the HPLC profiles of carnosic acid and of the dichloromethane plant surface extract of the fresh aerial parts of *Salvia somalensis*; Figure S9. Mycelial growth on potato dextrose agar (PDA) medium of *Colletotrichum coccodes*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Botrytis cinerea* used in the antifungal activity assay; Figure S10. Representative inhibition of growth of fungal mycelium after inoculation on poisoned media; Figure S11. Inhibition of growth of *Botrytis cinerea* (strain 2) mycelium 5 days after inoculation on poisoned media; Figure S12. Inhibition of growth of *Botrytis cinerea* (strain 7) mycelium 5 days after inoculation on poisoned media; Table S1. Compounds isolated from the dichloromethane extract of the fresh aerial parts of *S. somalensis*; Cultivation technique of *S. somalensis*. References [47,64–95,116] are also cited in Supplementary Materials.

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