



Article

Phytochemical Constituents and Antimicrobial Activity of *Euphorbia serrata* L. Extracts for *Borago officinalis* L. Crop Protection

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Abstract: The *Euphorbia* genus is the third-largest group of blooming plants, features a rich morphological variability, has a near-cosmopolitan distribution, and diverse medicinal uses. Nonetheless, phytochemical information about *Euphorbia serrata* L. extracts is not available. The objective of this research was to examine the constituents of the hydromethanolic extract of its aerial parts and propose valorization pathways. The results of gas chromatography-mass spectroscopy (GC–MS) demonstrated that 3-methylbutyl formate, quinic acid, N1-(4-hydroxybutyl)-N3-methylguanidine acetate, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one were the main phytochemicals, which have antimicrobial activity. Such activity was assayed against *Pseudomonas cichorii*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Sclerotinia sclerotiorum*, four of the most destructive diseases of borage (*Borago officinalis* L.) crops, obtaining minimum inhibitory concentrations (MICs) of 750 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ against the bacterium and the three fungal taxa, respectively, in in vitro tests. Conjugation of the extract with chitosan oligomers (COS) enhanced this activity, leading to MIC values of 187.5, 750, 500, and 500 $\mu\text{g}\cdot\text{mL}^{-1}$ for *P. cichorii*, *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum*, respectively. Additional in vivo assays against two of the pathogens confirmed the protective action of the COS–*E. serrata* extract conjugate complexes on artificially inoculated plants at a dose of 375 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ for *P. cichorii* and *F. oxysporum*, respectively. These findings suggest that this plant species can be a rich source of biorationals for prospective use in crop protection.

Keywords: bacterial blight; borage; cottony soft rot; *Fusarium* wilt; GC–MS; grey mold; phytochemicals; serrate spurge; crop protection



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1. Introduction

Euphorbia serrata L. is an arvense, annual plant that grows up to 0.7 m tall (Figure S1). It is known as serrate spurge or sawtooth spurge because of the serrate shape of the border of its leaves. Like other members of the family *Euphorbiaceae*, it is considered toxic and poisonous [1] because it exudes skin irritant and inflammatory, white milky latex (a complex emulsion consisting of starches, sugars, proteins, alkaloids, oils, tannins, resins, and gums, in which the presence of ingenol-3-palmitate has been documented [2]) when its stems or leaves are broken. However, according to Alves et al. [3], its medicinal use is documented in the treatment of conditions such as bronchitis, asthma, skin ailments, parasitic infections of the intestines, migraines, warts, gonorrhoea, and dysentery.

Reviews on this genus, such as those by Ernst et al. [4] and Jassbi [5], have emphasized the significance of investigating the therapeutic properties of *Euphorbia* species and their role as a natural product reservoir. For instance, phytochemical profiling of the leaves of *Euphorbia hirta* L. (employed in indigenous healing practices for diverse objectives, such as treating skin diseases, wounds, and rheumatism [6], and whose antimicrobial activity is supported by scientific evidence [7]) showed the presence of hydroxybenzoic and hydroxycinnamic acids, ethyl-gallic acid, digalloyl-quinic acid, feruloyl-coniferin, 5-*O*-caffeoyl-quinic acid trans isomer, protocatechuic acid-*O*-pentoside-*O*-hexoside, and di-, tri and tetra-*O*-galloyl-glucose isomers [8]. Various types of secondary metabolites including flavonoids, diterpenoids, triterpenoids, tannins, and lipids have been isolated and structurally characterized from *Euphorbia helioscopia* L., widely used in folk medicine in Turkey and China [9]. Yet, as far as the authors are aware, the composition of aerial parts of *E. serrata* has not been reported to date (only total phenolics and flavonoid contents are available [10], apart from the composition of its seeds [11]).

In light of the above, the aim of this study is two-fold: (i) to provide comprehensive information about *E. serrata* phytochemical composition and (ii) to explore prospective uses of its extracts. In particular, taking into consideration that phytochemicals present in other members of the family *Euphorbiaceae* have demonstrated strong activity against certain phytopathogens [12,13], its application to the control of plant diseases was investigated against four important pathogens of borage (*Borago officinalis* L.). The importance of this vegetable crop is increasing in Aragón (NE Spain) thanks to its promotion as part of avant-garde cookery in top-level restaurants, both in Spain and other countries. However, its production in the Ebro Valley is threatened by diseases like Fusarium wilt and root rot caused by *Fusarium oxysporum* Schltdl., which have been reported to destroy 70% of the crop in 2019 [14] and for which a control method has still not been found. Other two polyphagous pathogenic fungi, *Botrytis cinerea* Pers. (grey mold) and *Sclerotinia sclerotiorum* (Lib.) de Bary (cottony soft rot), either alone or in combination, also affect this specialty crop, particularly in open-air culture [15–17]. Concerning bacterial pathogens, *Pseudomonas cichorii* (Swingle 1925) Stapp 1928 [18] also poses a serious threat to borage, causing leaf spots, blight, and rotting, especially under high-humidity conditions such as those of glasshouse horticulture. Hence, the use of biorationals from *E. serrata* extracts to control these diseases would offer a natural-product-based approach to borage growers, meeting the specifications of Art. 14 of the European Directive 2009/128/EC, and may also find applicability in other vegetable crops also affected by these phytopathogens.

2. Material and Methods

2.1. Plant Samples and Reagents

Aerial parts from *E. serrata* plants were collected in May 2022 in the ‘*Vía Verde del Canfranero*’, near the city of Huesca, Spain (42°08′38.7″ N 0°26′54.0″ W), during the full blooming stage. Prof. J. Ascaso confirmed and validated a voucher specimen, which has been stored at the herbarium of the Escuela Politécnica Superior de Huesca, Universidad de Zaragoza. Samples from twenty specimens were combined to obtain representative composite samples, which were shade-dried, mechanically ground into powder, homogenized, and sieved through a 1 mm mesh.

Borage plants cv. “Movera” used in the in vivo experiments were supplied by Viveros Laraflor (Zaragoza, Spain).

Tryptic soy agar (TSA) and tryptic soy broth (TSB) were provided by Sigma–Aldrich Química (Madrid, Spain), while potato dextrose broth (PDB) and potato dextrose agar (PDA) were purchased from Becton Dickinson (Bergen County, NJ, USA). High-molecular-weight chitosan was procured from Hangzhou Simit Chem. and Tech. Co. (Hangzhou, China). Neutrase™ 0.8 L enzyme was acquired from Novozymes A/S (Bagsværd, Denmark).

2.2. Phytopathogens

Four *P. cichorii* bacterial strains (CITA Pci-2, CITA Pci-3, CITA Pci-4, and CITA Pci-5), obtained from diseased borage plants from the province of Zaragoza (Spain), were supplied by the Bacteriology Lab at the Center for Research and Agrifood Research and Technology Center of Aragón (Zaragoza, Spain) as subcultures on TSA. The fungal isolate of *B. cinerea* (CECT 20973) was obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). The fungal isolates of *F. oxysporum* (MYC-1593) (isolated from diseased borage plants) and *S. sclerotiorum* (MYC-799) were supplied by the Mycology Lab at the Center for Research and Agrifood Research and Technology Center of Aragón as subcultures on PDA.

2.3. Preparation of *E. serrata* Extract, Chitosan Oligomers, and Their Conjugate Complex

A dried aerial part sample (11.4 g) was combined with 90 mL of a methanol/water solution (1:17 v/v). The mixture was heated for 30 min at 50 °C, sonicated using a probe-type ultrasonicator (model UIP1000 hdT; Hielscher Ultrasonics; Teltow, Germany), and then centrifuged at 9000 rpm for 15 min. The resulting supernatant was filtered through Whatman No. 1 paper and subsequently subjected to freeze-drying to obtain the solid residue. The extraction yield was 52.6% (6 g). For subsequent gas chromatography-mass spectrophotometry (GC–MS) analysis, the freeze-dried extract was dissolved in HPLC-grade MeOH to obtain a 5 mg·mL⁻¹ solution, followed by further filtration.

The method described in [19], with the modifications specified in [20], was followed to obtain chitosan oligomers (COS) with a molecular weight < 2000 Da in a solution with a pH of 4.5. COS and *E. serrata* extract solutions were mixed in a 1:1 (v/v) ratio (150 mL of each solution, at a concentration of 3000 µg·mL⁻¹) and exposed to ultrasound for 15 min to form the conjugate complexes.

2.4. Physicochemical Characterization

A Thermo Scientific (Waltham, MA, USA) Nicolet iS50 Fourier-transform infrared (FTIR) spectrometer, with an in-built diamond attenuated total reflection (ATR) system, was used to register the infrared vibrational spectra of the shade-dried cyathia, shade-dried leaves, and latex samples. The spectral range of 400–4000 cm⁻¹ was scanned with a 1 cm⁻¹ resolution and 64 scans were co-added to generate the interferograms.

A gas chromatography-mass spectrometry (GC–MS) instrument comprising a model 7890A gas chromatograph coupled to a model 5975C quadrupole mass spectrometer (both from Agilent Technologies, Santa Clara, CA, USA) was used to elucidate the constituents of *E. serrata* aerial parts hydromethanolic extract. This analysis was outsourced to the Research Support Services (STI) at Universidad de Alicante (Alicante, Spain). The chromatographic conditions consisted of an injection volume of 1 µL, an injector temperature of 280 °C in splitless mode, an initial oven temperature of 60 °C held for 2 min, followed by a ramp of 10 °C·min⁻¹ up to a final temperature of 300 °C held for 15 min. An HP-5MS UI chromatographic column (30 m length, 0.250 mm diameter, and 0.25 µm film), also from Agilent Technologies, was employed for the separation of the compounds. The mass spectrometer's electron impact source and quadrupole temperatures were 230 and 150 °C, respectively, with an ionization energy of 70 eV. Components were identified through comparisons of mass spectra and retention times with those of authentic compounds and computer matching with the database of the National Institute of Standards and Technology (NIST11). Test mixture 2 for apolar capillary columns according to Grob (Supelco 86501) and PFTBA tuning standards were used for equipment calibration.

2.5. In Vitro Antimicrobial Activity Assessment

The antibacterial activity was assessed using the agar dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) standard M07-11 [21]. Initially, *P. cichorii* colonies were incubated at 28 °C for 24 h in TSB. Subsequently, serial dilutions were performed, starting from a concentration of 10⁸ CFU·mL⁻¹, to achieve a final inoculum of 10⁴ CFU·mL⁻¹. Next, the bacterial suspension was spread on the surface of TSA plates

to which the extract had been previously added, at concentrations ranging from 62.5 to 1500 $\mu\text{g}\cdot\text{mL}^{-1}$. The plates were then incubated at 28 °C for 24 h. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract at which no bacterial growth was visible. All experiments were replicated three times, with three plates per treatment and concentration.

The antifungal activity was determined using the agar dilution method as per the EUCAST antifungal susceptibility testing standard procedures [22]. Stock solution aliquots were incorporated into the PDA medium to produce final concentrations ranging from 62.5 to 1500 $\mu\text{g}\cdot\text{mL}^{-1}$. Mycelial plugs ($\varnothing = 5$ mm), from the margin of 7-day-old PDA cultures of *B. cinerea*, *S. sclerotiorum*, and *F. oxysporum* were transferred to the center of PDA plates amended with the aforementioned concentrations (three plates per treatment and concentration, with two duplicates). Plates were incubated in the dark at 25 °C for seven days. The control consisted in replacing the extract with the solvent used for extraction (i.e., methanol:water 1:17 v/v) in the PDA medium. The inhibition of mycelial growth was estimated according to the formula in Equation (1):

$$((d_c - d_t)/d_c) \times 100, \quad (1)$$

where d_c and d_t represent the mean diameters of the control and treated fungal colonies, respectively. The effective concentrations (EC₅₀ and EC₉₀) were determined via PROBIT analysis in SPSS Statistics v. 25 (IBM, New York, NY, USA). The synergy factor (SF), which measures the degree of interaction, was estimated using Wadley's method [23].

Since the Shapiro–Wilk and Levene tests indicated that the homogeneity and homoscedasticity requirements were fulfilled, the mycelial growth inhibition results were subjected to one-way analysis of variance (ANOVA) and subsequent post hoc comparison of means through Tukey's test at a significance level of $p < 0.05$ in IBM SPSS Statistics v. 25.

2.6. In Vivo Tests on Borage

Borage plants cv. "Movera" were used for in vivo experiments to assess the efficacy of the most potent in vitro treatment (i.e., COS-*E. serrata*) against artificially inoculated *P. cichorii* and *F. oxysporum*, following the methods described in [24] and [14], respectively, with minor modifications. Multiple 4 × 4 cm pots were used to grow the borage plants with sterile peat as substrate, with 5 plants per treatment and pathogen; two independent replicates were conducted. The treatment was applied at two concentrations (MIC and MIC × 2, i.e., 187.5 and 375 $\mu\text{g}\cdot\text{mL}^{-1}$ for *P. cichorii*, and 500 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ for *F. oxysporum*). In the case of *P. cichorii*, it was applied through spraying (3 mL per plant). After 2 h, an isolated colony of *P. cichorii* was inoculated into the youngest leaflets by making 3–4 punctures using a sterile entomological pin. For the *F. oxysporum* assay, the fungus was previously grown in 250 mL flasks containing PDB for 3 days at 25 °C in the dark with constant shaking. Borage roots were then dipped in a suspension of 6×10^6 conidia·mL⁻¹ for 2 min, and the plants were transferred to plastic pots with sterilized substrate. Non-inoculated plants sprayed with sterilized water or dipped in sterilized water were used as negative controls. All plants were incubated in a growth chamber (25 °C, 16/8 h photoperiod) for ten days.

3. Results

3.1. Infrared Spectra

The main absorption bands in the FTIR spectra (Figure S2) of *E. serrata* inflorescences (cyathia), leaves, and latex are listed in Table 1, alongside their functional group assignments. The symmetric C–H stretching vibrations of aliphatic groups (latex bands) are observed at 2916 ± 1 and 2848 ± 1 cm⁻¹, while the C=O stretching band at 1730 ± 2 cm⁻¹ indicates ester bonds and carboxylic acid groups. In the fingerprint region of the spectrum, C–H and O–H deformation vibrations characteristic of carbohydrates can be observed between 1200 and 1462 cm⁻¹. Different types of C–H, C–O, and CH₃ vibrations, which cannot be identified more precisely, appear in the lower range of the fingerprint region below 1200 cm⁻¹ [25].

Table 1. Main absorption bands in the FTIR spectra of *Euphorbia serrata* plant organs and latex.

Flowers	Leaves	Latex	Assignment
3300	3297	3355	Bonded O–H stretching (cellulose, hemicellulose, lignin)
2917	2916	2916	–CH ₂ asymmetric stretching of alkyls (cutine, wax, pectin)
2849	2848	2848	–CH ₂ symmetric stretching (cutine and wax); CH ₂ –(C6)–bending (cellulose)
1732	1732	1728	C=O from esters
1635	1635	1646	C=O stretching of alkyl ester
	1598		phenyl ring (aromatic skeletal vibration)
	1472	1472	methyl C–H asymmetrical
1462	1462	1463	scissoring mode of –CH in CH ₂
1416	1416	1417	(CH)C=CH ₂ in plane scissoring
1362	1375	1374	C–C asymmetrical stretching phenolic hydroxyl groups
1311	1315	1312	C–H in-plane bending
1243	1245	1243	C–O stretching; amide N–H vibration
1170	1169	1167	C–O–C asymmetric stretching in cellulose; C–C in-plane
1103	1101	1103	C–O–C stretching in the pyranose skeletal ring
1050	1062	1050	stretching mode of C–O bond
1019	1019	1019	C–H bending; C–C stretching
	959	961	C–H out-of-plane bending
719	719	719	CH ₂ rocking vibrations

Since the functional groups were found to be similar in the three organs (cyathia, leaves, and latex), a combined sample was utilized for the rest of the study. This approach is advantageous from a practical standpoint as it obviates the necessity of separating the aerial plant organs for subsequent extraction.

3.2. GC–MS Characterization of the Extract

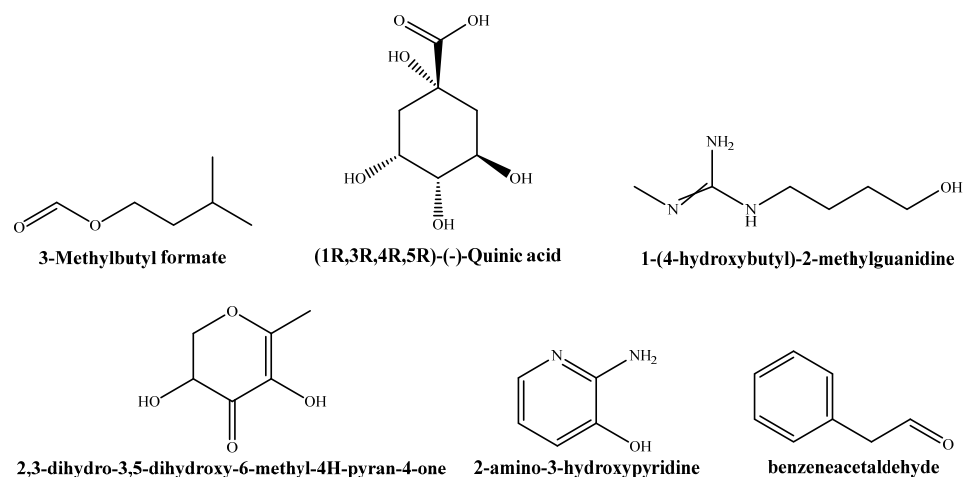
Among the fifty-three phytochemicals identified (Table 2, Figure 1 and Figure S3) in *E. serrata* aerial parts extract, the most abundant were: 1-butanol, 3-methyl-, formate (or 3-methylbutyl formate) (15.5%); quinic acid (14.4%), N1-(4-hydroxybutyl)-N3-methylguanidine acetate (5.3%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (or DDMP) (4%), 2-amino-3-hydroxypyridine or 2-amino-3-pyridinol (3.9%), 4-oxohexenal (3.4%), ethyl methyl acetal acetone (3.2%), benzeneacetaldehyde (3.2%), squalene (2.7%), and hexadecanoic acid (1.9%). Among them, the assignments of (E)-4-oxohex-2-enal and ethyl methyl acetal acetone had quality of resemblance (Qual) values below 80, so their assignment is dubious.

Table 2. Main phytoconstituents identified by GC–MS in *Euphorbia serrata* aerial parts extract.

RT (min)	Area (%)	Assignment
4.2767	1.8852	1,3-Dihydroxyacetone dimer
4.3539	1.2792	Dihydroxyacetone
4.5853	0.8633	Butyrolactone
4.6625	0.9757	Formic acid, pentyl ester
4.7337	1.1785	2-Cyclopenten-1-one, 2-hydroxy-
5.0602	0.7557	2H-Pyran-2-one
5.5528	0.7291	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
5.7308	1.3414	Formic acid, 2-propenyl ester
5.7902	0.7461	Cyclopropanecarboxamide
5.8614	3.3739	(E)-4-Oxohex-2-enal
6.4609	0.6257	Guanazine
6.5499	3.1997	Benzeneacetaldehyde
6.728	0.8216	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
7.3393	1.0234	2-Heptanamine, 5-methyl-
7.5233	15.4575	1-Butanol, 3-methyl, formate

Table 2. Cont.

RT (min)	Area (%)	Assignment
7.9566	1.2667	Ethanamine, N-ethyl-N-nitroso-
8.0871	3.9711	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
8.3898	0.7566	2(3H)-Furanone, dihydro-4-hydroxy-
8.4789	1.0122	Bicyclo[4.2.0]octa-1,3,5-triene, 7-(2-propenyl)-
8.9181	1.7303	Catechol
9.0487	1.6384	3-(α -Hydroxyethyl)-aniline
9.1674	1.22	Benzofuran, 2,3-dihydro-
9.3573	0.5829	5-Hydroxymethylfurfural
9.5888	0.6945	Butanoic acid, 3-oxo-, hexyl ester
9.8381	0.9602	1,2-Benzenediol, 3-methoxy-
10.0636	0.4961	Hydroquinone
10.1645	0.5023	Isosorbide
10.2239	1.3233	2-Butanone, 4-hydroxy-3-methyl-
10.5562	1.5294	2-Methoxy-4-vinylphenol
11.4762	0.3876	1,4-Hexadiene, 2,3,4,5-tetramethyl-
11.6543	0.4644	N,N-Diethylaniline
11.8679	0.2927	6-Methylthiocarbonyl-2,4-diamino-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidin
12.4615	5.2679	N1-(4-hydroxybutyl)-N3-methylguanidine acetate
12.4852	3.2235	Acetone, ethyl methyl acetal
12.8235	0.8293	Ethanone, 1-(3-hydroxy-4-methoxyphenyl)-
12.8591	1.065	Adamantane
13.0491	1.0355	Buformin
13.1737	1.1752	2-Hydroxy-1-(1'-pyrrolidyl)-1-buten-3-one
13.9572	0.2278	3-Methyl-4-phenyl-1H-pyrrole
14.4142	11.7827	(1R,3R,4R,5R)-(-)-Quinic acid
15.8802	3.9341	2-Amino-3-hydroxypyridine
16.0286	0.7147	d-Proline, N-allyloxycarbonyl-, allyl ester
16.907	1.3827	Tetradecanoic acid
17.5896	0.5213	Hexadecanoic acid, methyl ester
17.7202	0.6145	9-Hexadecenoic acid
17.916	1.9156	n-Hexadecanoic acid
19.2811	0.593	Methyl 6,9,12-hexadecatrienoate
19.4948	0.1775	Heptadecanoic acid, 16-methyl-, methyl ester
19.5898	0.4676	9-Octadecenoic acid, (E)-
19.7856	0.3783	Octadecanoic acid
25.0918	2.7381	Squalene
25.5251	1.7309	n-Tetracosanol-1
30.9203	1.7537	N-Methyl-1-adamantaneacetamide

Figure 1. Main phytochemicals identified in *Euphorbia serrata* extract.

3.3. In Vitro Antimicrobial Activity

3.3.1. In Vitro Antibacterial Activity

Some differences in terms of sensitivity to COS were observed among the four *P. cichorii* strains, with MIC values ranging from 500 to 750 $\mu\text{g}\cdot\text{mL}^{-1}$, as shown in Table 3. However, the MIC values obtained for *E. serrata* extract (comparable to/slightly higher than those of COS) and the COS–*E. serrata* conjugate complex (substantially lower than those of COS and *E. serrata*, with SF values > 1) were the same for all four strains.

Table 3. Minimum inhibitory concentrations (in $\mu\text{g}\cdot\text{mL}^{-1}$) against four *P. cichorii* strains of chitosan oligomers (COS) and *E. serrata* aerial part extract alone, and their conjugate complex (COS–*E. serrata*). Synergy factors (SF) calculated for the conjugate complex are also indicated.

Bacteria	Strain	COS	<i>E. serrata</i>	COS– <i>E. serrata</i>	SF
<i>P. cichorii</i>	Pci-2	750	750	187.5	4.00
	Pci-3	750	750	187.5	4.00
	Pci-4	500	750	187.5	3.20
	Pci-5	500	750	187.5	3.20

3.3.2. In Vitro Antifungal Activity

Figure S4 summarizes the results of the antifungal capacity testing. Higher doses resulted in reduced radial mycelium growth for the three tested treatments (viz., COS, *E. serrata* extract, and COS–*E. serrata* extract conjugate complex), with statistically significant differences in the case of the three pathogens. COS fully inhibited the mycelial growth of *B. cinerea* and *S. sclerotiorum* at 1500 $\mu\text{g}\cdot\text{mL}^{-1}$ and that of *F. oxysporum* at 1000 $\mu\text{g}\cdot\text{mL}^{-1}$. *Euphorbia serrata* extract was more effective, resulting in complete inhibition of the three pathogens at 1000 $\mu\text{g}\cdot\text{mL}^{-1}$. However, the antifungal activity was substantially increased by the application of conjugate complexes: the COS–*E. serrata* conjugate complex led to full inhibition at doses between 500 and 750 $\mu\text{g}\cdot\text{mL}^{-1}$. Such activity enhancement was quantified using the effective concentration (EC) values of the separate bioactive products and those of the conjugate complexes (Table 4), obtaining the SF summarized in Table 5. Given that values close to two were obtained against the three phytopathogens, a clear synergistic action between COS and the extract may be inferred also in terms of antifungal activity.

Table 4. Effective concentrations (EC, in $\mu\text{g}\cdot\text{mL}^{-1}$) against *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum* of chitosan oligomers (COS) and *E. serrata* aerial part extract alone, and their conjugate complex (COS–*E. serrata*).

Treatment	EC	<i>B. cinerea</i>	<i>F. oxysporum</i>	<i>S. sclerotiorum</i>
COS	EC ₅₀	236.2	233.2	864.3
	EC ₉₀	1426.3	743.4	1344.8
<i>E. serrata</i>	EC ₅₀	402.2	402.1	408.7
	EC ₉₀	832.4	796.2	673.4
COS– <i>E. serrata</i>	EC ₅₀	140.0	142.4	282.0
	EC ₉₀	500.6	378.4	363.7

Table 5. Synergy factors for the COS–*E. serrata* conjugate complex.

Treatment	EC	<i>B. cinerea</i>	<i>F. oxysporum</i>	<i>S. sclerotiorum</i>
COS– <i>E. serrata</i>	EC ₅₀	2.13	2.07	1.97
	EC ₉₀	2.10	2.03	2.47

3.4. In Vivo Antimicrobial Activity

3.4.1. In Vivo Antibacterial Activity against *P. cichorii*

Taking into consideration that the COS-*E. serrata* conjugate complex was shown to be the most active in the in vitro tests, its bacteriostatic activity was further tested in in vivo tests. It was applied as a preventive treatment against bacterial blight on borage plants cv. “Movera” (as shown in Figure 2). Ten days post-inoculation, positive control plants (inoculated with *P. cichorii* only) displayed necrotic lesions that covered the entire leaf (Figure 2b). Plants sprayed with the COS-*E. serrata* conjugate complex at a concentration equal to the MIC ($187.5 \mu\text{g}\cdot\text{mL}^{-1}$) had necrotic lesions in the area where the pathogen was inoculated, which did not spread to the entire leaf, but showed some discoloration around these necrotic spots (Figure 2c). In turn, plants treated with a dose twice the MIC ($375 \mu\text{g}\cdot\text{mL}^{-1}$) showed barely any signs of disease attack (as shown in Figure 2d). No symptoms were observed in mock-inoculated plants (sprayed with sterile distilled water) (Figure 2a).

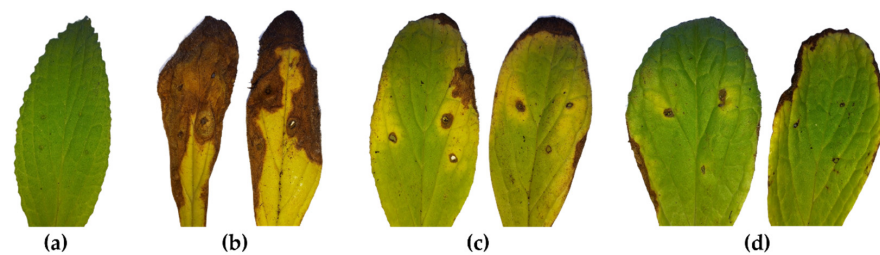


Figure 2. Symptoms of bacterial blight in borage plants ten days post-inoculation in (a) negative control plants; (b) positive control plants; (c) plants sprayed with COS-*E. serrata* conjugate complex at $187.5 \mu\text{g}\cdot\text{mL}^{-1}$; and (d) plants sprayed with COS-*E. serrata* conjugate complex at $375 \mu\text{g}\cdot\text{mL}^{-1}$.

3.4.2. In Vivo Antifungal Activity against *F. oxysporum*

Figure 3 shows the results of the in vivo assays conducted with COS-*E. serrata* conjugate complex on borage cv. “Movera” plants to assess the protective (fungistatic) activity against *Fusarium* wilt. All positive control plants showed the characteristic severe wilting and yellowing symptoms, accompanied by dry necrosis of the central veins of some leaves, followed by plant death (Figure 3b). The disease incidence among plants treated with the extract at a concentration equal to the MIC ($500 \mu\text{g}\cdot\text{mL}^{-1}$) was high (80%), but the disease severity was lower (Figure 3c). To achieve complete protection, the dose had to be increased to $1000 \mu\text{g}\cdot\text{mL}^{-1}$: at twice the MIC, after which all plants remained asymptomatic (Figure 3d). It should be noted that at the highest concentration tested ($1000 \mu\text{g}\cdot\text{mL}^{-1}$), borage plants did not display symptoms of phytotoxicity, with no visual differences among plants treated at $1000 \mu\text{g}\cdot\text{mL}^{-1}$ and the negative control (Figure 3a).

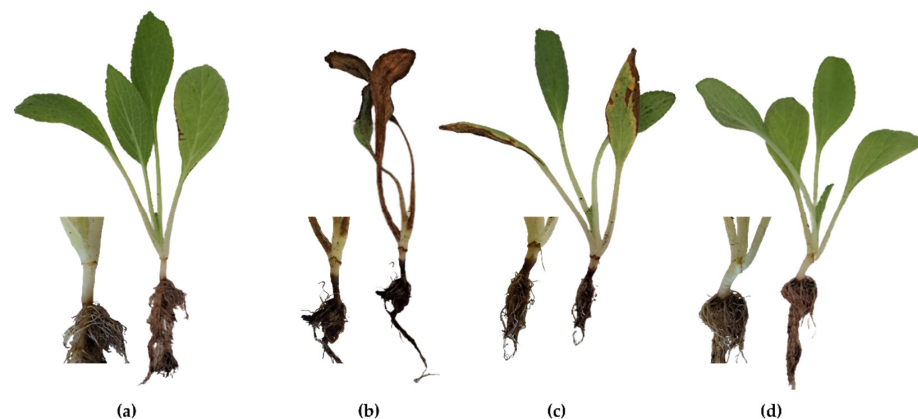


Figure 3. Symptoms of wilt, stem, and root rot produced by *F. oxysporum* in borage plants ten days post-inoculation in (a) negative control plants; (b) positive control plants; (c) plants treated with COS-*E. serrata* conjugate complex at $500 \mu\text{g}\cdot\text{mL}^{-1}$; and (d) plants treated with COS-*E. serrata* conjugate complex at $1000 \mu\text{g}\cdot\text{mL}^{-1}$.

4. Discussion

4.1. On the Phytochemical Profile of the Extract

3-Methylbutyl formate is a wax monoester related to 2-methylbutyl esters found in *Chamaemelum nobile* (L.) All. It was previously identified in red raspberries [26]. Meanwhile, its analogs, 3-methylbutyl acetate and 3-methylbutyl propionate, were identified in yeasts inhabiting *Prunus avium* (L.) L. and *Prunus cerasus* L. [27]. These volatile organic compounds have the potential for post-harvest disease management, and their emission is a predominant antifungal mechanism of antagonistic microorganisms. Ruiz-Moyano et al. [28] found that 2-methylbutyl acetate, produced by *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak and Phaff ex M.T. Sm., effectively controlled the incidence of *B. cinerea* in strawberries and cherries. Additionally, 3-methylbutyl hexanoate and 3-methylbutyl pentanoate from *Candida sake* (Saito and Oda) van Uden and H.R. Buckley reduced the disease incidence of *Penicillium expansum* Link on apple fruits by 53% and the disease severity by 20% [29,30]. Thus, it is expected that 3-methylbutyl esters from *E. serrata* can induce intracellular ROS accumulation and inhibit mycelia growth and conidial sporulation, as demonstrated for other 3-methylbutyl esters against *P. expansum*, *Curvularia lunata* (Wakker) Boedijn, *B. cinerea*, and *Alternaria alternata* (Fr.) Keissl. [30].

Concerning quinic acid, this cyclohexanecarboxylic acid has been reported in the bark of *Uncaria tomentosa* (Willd. ex Schult.) DC. [31], as well as in the extracts of several parts of medicinal plants, including *Achillea pseudoaleppica* Hub.-Mor., *Artemisia annua* L., *Coffea arabica* L., *Haematocarpus Validus* (Miers) Bakh.fil. ex Forman, *Hypericum empetrifolium* Willd., *Phagnalon saxatile* (L.) Cass., *Rumex nepalensis*, and *Ziziphus lotus* L. [32]. Bai et al. [33] suggested that it could be used as an antibacterial agent in food preservation. The modulation of ribosome function, aminoacyl-tRNA synthesis, and alterations in the levels of glycerophospholipids and fatty acids, as well as interference with membrane fluidity by disrupting the oxidative phosphorylation pathway, are all significant contributors to its antibacterial effect [32]. Research on cellular functions has revealed that quinic acid leads to a notable decline in intracellular pH, lowers succinate dehydrogenase activity, and results in reduced intracellular ATP concentration. Lu et al. [34] indicated that the binding of quinic acid to RhlA, RhlR, and RhlB receptors interferes with the binding of signal molecules to these receptors, leading to the transcriptional regulation of signaling pathways.

1-(4-hydroxybutyl)-2-methylguanidine acetate is the main component of *Eucalyptus grandis* W.Hill ex Maiden leaves [35] and has also been identified in the fresh extract of *Cucumis sativus* L. [36] and in fiddleheads (fern fronds) [37]. Its origin may be related to the hydrolysis of crambescin A2 (the specific constituent of *Crambe abyssinica* Hochst. ex R.E.Fr.), demethylation of 3-ethyl-1-(2-ethyl-4-hydroxybutyl)guanidine, or deamination of *L*-arginine (Figure 4).

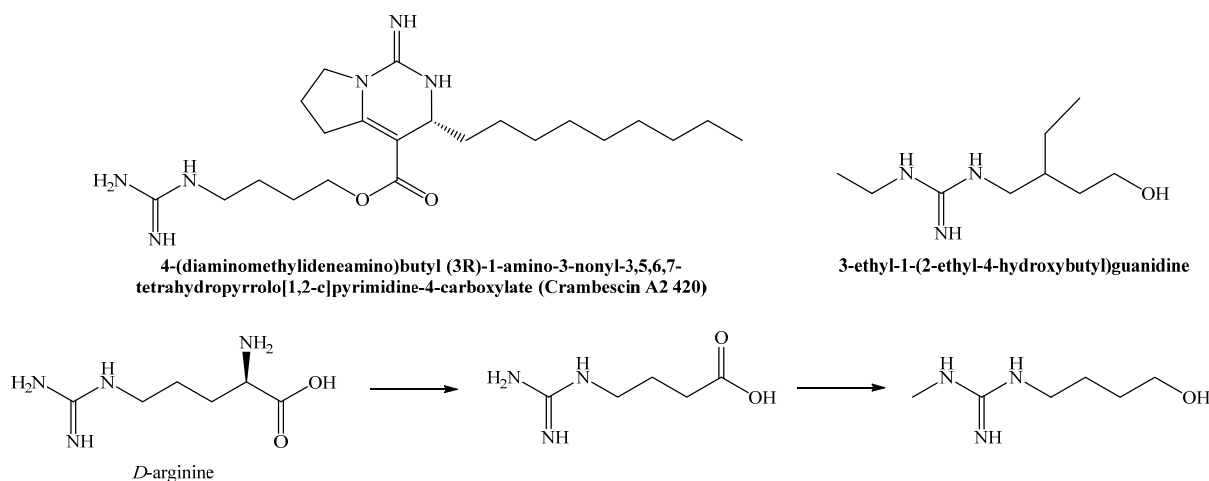


Figure 4. (top) 1-(4-hydroxybutyl)-2-methylguanidine precursors: crambescin A and 3-ethyl-1-(2-ethyl-4-hydroxybutyl)guanidine; (bottom) Scheme on the deamination of *L*-arginine.

DDMP or 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one is a strong antioxidant in glucose–histidine Maillard reaction products [38]. It is also present in the extracts from *Acalypha indica* L., *Ammannia baccifera* L., *Borassus flabellifer* L., *Camellia japonica* L., *Cocculus hirsutus* (L.) Diels, *C. sativus*, *Leucas aspera* (Willd.) Link, *Litchi chinensis* Sonn., *Marsilea quadrifolia* L., *Punica granatum* L., *Sambucus nigra* L., and *Rumex vesicarius* L. [39–41]. Antibacterial activity has been demonstrated against *Erwinia amylovora* (Burrill 1882) Winslow et al., 1920 and *Xylophilus ampelinus* (Panagopoulos 1969) Willems et al., 1987 [39], and strong antifungal activity has been reported against *Verticillium dahliae* Kleb., *Diaporthe amygdali* (Delacr.) Udayanga, Crous and K.D. Hyde, and *Phytophthora megasperma* Drechsler [40].

2-amino-3-hydroxypyridine or 2-amino-3-pyridinol is a natural dye used for manufacturing hair colors, present in *Petroselinum crispum* (Mill) Fuss. and *Coriandrum sativum* L. [42]. It has been found biologically important in the preparation of clinical anti-inflammatory analgesics [43].

Benzeneacetaldehyde, previously identified in *Armeria maritima* (Mill.) Willd. flowers [44], has shown antibacterial activity [45].

Squalene is a triterpene with lipophilic properties and serves as a precursor to ergosterol, an important component of the plasmatic membrane in fungi and yeasts. Prior research has shown squalene to be a significant phytochemical in the bark of *Quercus ilex* subsp. *ballota* (Desf.) Samp. [46] and leaves of *Hibiscus syriacus* L. [47]. Furthermore, it is understood that squalene accumulation inside cells can lead to the formation of vesicles that contain squalene, thereby disrupting the fungal cell membrane. This process consequently removes essential membrane lipid components, weakening the fungal cells [48].

Hexadecanoic acid (or palmitic acid), found as the major component in *A. maritima* [44], has been shown to be highly suitable for the integrated control of phytopathogens [49].

Concerning the two constituents with dubious assignments (i.e., with low Qual values), 4-oxohexanal has not been previously identified in plants (only its analog 2-isopropyl-5-oxohexanal) [50]. It is a precursor of the gamma-lactone named 4-oxanolide, a metabolite produced by *Saccharomyces cerevisiae* (Desm.) Meyen. As for ethyl methyl acetal acetone (or 2-ethoxy-2-methoxypropane), it has not previously been reported in plant extracts either, but its derivative, 1-chloro-2-ethoxy-2-methoxy-propane has been reported from *Ehretia laevis* Roxb. [51] and *Maytenus emarginata* Willd. [52]. Hence, caution is advised as identification of these compounds is probably unreliable.

It is worth noting that no correspondence between the main phytochemicals reported above and the phytoconstituents reported for *E. hirta* leaves extract by Mekam et al. [8] was observed. This difference is tentatively ascribed to the different analytical methods employed (GC–MS vs. LC–ESI–IT–MS/MS).

4.2. On the Antimicrobial Activity in Comparison with Other *Euphorbia* spp. Extracts

Concerning previous reports on the antimicrobial activity of *E. serrata*, they are limited to human pathogens. Alghazeer et al. [10] found MIC values in the range of 3.13–6.25 mg·mL^{−1} against *Staphylococcus aureus* Rosenbach, 1884, methicillin-resistant *S. aureus*, *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919, and *Salmonella typhi* (Schroeter, 1886) Warren and Scott, 1930 for methanolic extracts. According to Daud et al. [53], methanolic and chloroform extracts of *E. serrata* also inhibited the growth of *Enterococcus faecalis* (Andrewes and Horder, 1906), Schleifer and Kilpper-Bälz, 1984, *Klebsiella pneumoniae* (Schroeter, 1886) Trevisan, 1887, and *Pseudomonas aeruginosa* (Schroeter, 1872) Migula, 1900 (apart from those of *S. aureus*, *E. coli*, and *S. typhi*), but they did not report the MIC values.

With regard to the activity of other *Euphorbia* spp. extracts tested against human and plant pathogens, a summary of the efficacies reported in the literature is presented in Table 6. It should be noted that direct comparisons are not possible because the pathogens are different, and, in those cases in which the same species were tested, the strains were different. Furthermore, results were not expressed as EC or as MIC values in all studies. Consequently, the comparisons presented below should be interpreted as a first approximation. Focusing on the data reported against phytopathogens, the antibacterial activity of the

non-conjugated *E. serrata* extract would be comparable to that of *Euphorbia cotinifolia* L. [54]. As for its antifungal activity, it would be substantially higher than those of *E. hirta* and *Euphorbia tirucalli* L. against *F. oxysporum* [8,55,56] and higher than those of *Euphorbia guyoniana* Boiss. and Reut. and *Euphorbia royleana* Boiss. against other *Fusarium* spp. [57,58], while it would be comparable to that of *Euphorbia macroclada* Boiss. (for which full inhibition of *F. oxysporum* was reported at 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ for chloroform, petroleum ether, and butanol flower extracts, as well as for a chloroform stem extract) [59].

Table 6. Inhibition values reported in the literature for *Euphorbia* spp. extracts against various microorganisms.

<i>Euphorbia</i> spp.	Extraction Medium	Pathogens	Efficacy	Ref.
<i>E. guyoniana</i>	water (20%)	<i>Fusarium graminearum</i> <i>Fusarium sporotrichioides</i>	IZ = 27.3–47.85 mm	[57]
<i>E. cotinifolia</i>	ethyl acetate and methanol	<i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> <i>Xanthomonas axonopodis</i> pv. <i>vesicatoria</i> <i>Xanthomonas oryzae</i> <i>Agrobacterium tumefaciens</i> <i>Xanthomonas campestris</i> pv. <i>campestris</i> <i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i> <i>Pseudomonas solanacearum</i> <i>Pseudomonas syringae</i>	MIC = 300–1300 $\mu\text{g}\cdot\text{mL}^{-1}$	[54]
<i>E. royleana</i>	methanol, hexane, and water (800 $\mu\text{g}\cdot\text{mL}^{-1}$)	<i>Escherichia coli</i> <i>Bacillus subtilis</i> <i>Pasteurella multocida</i> <i>Aspergillus niger</i> <i>Fusarium solani</i>	IZ = 5.7–14.0 mm	[58]
<i>E. macroclada</i>	butanol, chloroform, petroleum ether, and water extracts of flowers, stems, and leaves (1000 $\mu\text{g}\cdot\text{mL}^{-1}$)	<i>Fusarium oxysporum</i> <i>Rhizoctonia solani</i> <i>Pythium</i> sp. <i>Verticillium dahliae</i> <i>Alternaria solani</i> <i>Stemphylium solani</i> <i>Rhizopus stolonifer</i> <i>Penicillium italicum</i> <i>Cladosporium</i> sp. <i>Mucor</i> sp.	IR = 0.1–89.9% (pooled avg.) for flower extracts; 7–81.3% for stem extracts; 0–8.1% for leaf extracts	[59]
<i>E. aleppica</i> <i>E. szovitsii</i> <i>E. falcata</i> <i>E. denticulata</i> <i>E. macroclada</i> <i>E. cheiradenia</i> <i>E. virgata</i> <i>E. petiolata</i>	methanol	<i>Staphylococcus aureus</i> <i>Bacillus megaterium</i> <i>Proteus vulgaris</i> <i>Klebsiella pneumoniae</i> <i>E. coli</i> <i>Pseudomonas aeruginosa</i> <i>Candida albicans</i> <i>Candida glabrata</i> <i>Candida tropicalis</i> <i>Trichophyton</i> sp. <i>Epidermophyton</i> sp.	IZ = n.a.–25 mm	[60]
<i>E. balsamifera</i>	ethanol, petroleum ether, chloroform, and water	<i>Salmonella typhimurium</i> <i>P. aeruginosa</i> <i>Klebsiella</i> spp. <i>E. coli</i> <i>C. albicans</i>	MIC = 5000–6000 $\mu\text{g}\cdot\text{mL}^{-1}$ MBC = 4500–6000 $\mu\text{g}\cdot\text{mL}^{-1}$	[61]

Table 6. Cont.

<i>Euphorbia</i> spp.	Extraction Medium	Pathogens	Efficacy	Ref.
<i>E. granulata</i> <i>E. helioscopia</i> <i>E. hirta</i>	ethanol	<i>E. coli</i> <i>K. pneumoniae</i> <i>P. vulgaris</i> <i>S. aureus</i> <i>Staphylococcus epidermidis</i> <i>Streptococcus pyogenes</i> <i>Aspergillus fumigatus</i> <i>C. albicans</i> <i>C. tropicalis</i> <i>Geotrichum candidum</i> <i>Microsporium canis</i> <i>Trichophyton mentagrophytes</i>	MIC = 3.90–250 $\mu\text{g}\cdot\text{mL}^{-1}$ for Gram-negative bacteria; 1.95–15.62 $\mu\text{g}\cdot\text{mL}^{-1}$ for Gram-positive bacteria; 1.95–500 $\mu\text{g}\cdot\text{mL}^{-1}$ for fungi	[62]
<i>E. hirta</i> <i>E. tirucalli</i>	ethanol (concentration not available)	<i>Colletotrichum capsicii</i> <i>Fusarium pallidroseum</i> <i>Botryodiplodia theobromae</i> <i>Alternaria alternata</i> <i>Penicillium citrinum</i> <i>Phomopsis caricae-papayae</i> <i>A. niger</i>	IZ = n.a.–9 mm	[63]
	ethanol and water	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> <i>A. solani</i> <i>R. solani</i>	IC ₅₀ = 2930–32,140 $\mu\text{g}\cdot\text{mL}^{-1}$	[8]
	water (5%)	<i>R. solani</i> <i>F. oxysporum</i> <i>Macrophomina phaseolina</i>	IR = 10–80%	[55]
	ethanol (concentration not available)	<i>S. aureus</i> <i>Bacillus cereus</i> <i>Salmonella typhi</i> <i>K. pneumoniae</i> <i>P. aeruginosa</i> <i>A. niger</i> <i>A. fumigatus</i> <i>Aspergillus flavus</i> <i>Rhizopus oryzae</i>	IZ = n.a.–13.90 mm	[64]
<i>E. hirta</i>	methanol	<i>S. aureus</i> <i>Bacillus thuringiensis</i> <i>B. subtilis</i> <i>Micrococcus</i> sp. <i>E. coli</i> <i>K. pneumoniae</i> <i>Proteus mirabilis</i> <i>S. typhi</i> <i>C. albicans</i>	IZ = n.a.–29 mm, at 100,000 $\mu\text{g}\cdot\text{mL}^{-1}$ MIC = 3130–100,000 $\mu\text{g}\cdot\text{mL}^{-1}$ MBC/ MFC = 3130–100,000 $\mu\text{g}\cdot\text{mL}^{-1}$	[7]
	methanol, petroleum ether, ethyl ether, and ethyl acetate	<i>E. coli</i> <i>P. aeruginosa</i> <i>P. mirabilis</i> <i>S. aureus</i> <i>A. flavus</i> <i>A. niger</i> <i>T. mentagrophytes</i> <i>C. albicans</i>	MIC = n.a.–625 $\mu\text{g}\cdot\text{mL}^{-1}$ MBC/ MFC = n.a.–1250 $\mu\text{g}\cdot\text{mL}^{-1}$	[65]

Table 6. Cont.

<i>Euphorbia</i> spp.	Extraction Medium	Pathogens	Efficacy	Ref.
	acetone, methanol, and water	<i>S. aureus</i> <i>S. epidermidis</i> <i>Enterococcus faecalis</i> <i>E. coli</i> <i>P. aeruginosa</i>	IZ = n.a.–16 mm, at 10,000 $\mu\text{g}\cdot\text{mL}^{-1}$ MIC = n.a.–400 $\mu\text{g}\cdot\text{mL}^{-1}$	[66]
<i>E. tirucalli</i>	methanol	<i>E. coli</i> <i>P. vulgaris</i> <i>Salmonella enteritidis</i> <i>B. subtilis</i> <i>S. aureus</i> <i>P. aeruginosa</i> <i>K. pneumoniae</i> , <i>C. albicans</i> <i>C. tropicalis</i> <i>A. niger</i> <i>A. fumigatus</i> <i>A. flavus</i> <i>F. oxysporum</i>	IZ = 1–21.5 mm, at 7500 $\mu\text{g}\cdot\text{mL}^{-1}$ MIC = n.a.–>1000 $\mu\text{g}\cdot\text{mL}^{-1}$	[56]
<i>E. macrorrhiza</i>	butanol, chloroform, ethyl acetate, methanol, hexane	<i>S. aureus</i> <i>E. coli</i> <i>C. albicans</i>	MIC = 500–>2000 $\mu\text{g}\cdot\text{mL}^{-1}$ MBC/ MFC = 1000–>2000 $\mu\text{g}\cdot\text{mL}^{-1}$	[67]
<i>E. neriifolia</i>	butanol, chloroform, ethyl acetate, ethanol, and water (50 $\mu\text{g}\cdot\text{mL}^{-1}$)	<i>S. aureus</i> <i>K. pneumoniae</i> <i>E. coli</i> <i>P. vulgaris</i> <i>Pseudomonas fluorescens</i>	IZ = n.a.–8 mm	[68]
<i>E. helioscopia</i>	ethanol and water (1000 $\mu\text{g}\cdot\text{mL}^{-1}$)	<i>Trichoderma harzianum</i> <i>Rhizopus nigricans</i> <i>A. niger</i>	IZ = 32–33 mm IZ = 22–32 mm IZ = 24–34 mm	[69]

IZ: inhibition zone; MIC: minimum inhibitory concentration; IR: inhibition rate; MBC: Minimum Bactericidal Concentration; IC₅₀: half-maximal inhibitory concentration; MFC: minimum fungicidal concentration; n.a.: no activity.

4.3. On the Efficacy of Other Natural Compounds against the Phytopathogens under Study

When comparing the efficacy of *E. serrata* extract against other plant extracts tested against the four phytopathogens considered here (Table S1) [24,44,70–102], it may be observed that the MIC values (750 $\mu\text{g}\cdot\text{mL}^{-1}$ against *P. cichorii* and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ against the three fungal taxa) are among the lowest reported to date. The antibacterial activity against *P. cichorii* is the highest among the plant extracts presented in Table S1, comparable to that of *Ginkgo biloba* L. [24]. In the case of *B. cinerea*, the efficacy is only lower than those of *U. tomentosa* (375 $\mu\text{g}\cdot\text{mL}^{-1}$) [74], *Cinnamomum cassia* (L.) J.Presl, *Syzygium aromaticum* (L.) Merr. and Perry, and *Origanum syriacum* L. (600 $\mu\text{g}\cdot\text{mL}^{-1}$) [78,79,82], and it is comparable to that of *Silene uniflora* Roth (1000 $\mu\text{g}\cdot\text{mL}^{-1}$) [73]. Against *F. oxysporum* spp., the activity is lower than those of *Cestrum nocturnum* L. ethyl acetate and methanol extracts (500 $\mu\text{g}\cdot\text{mL}^{-1}$) [89] and *A. maritima* extract (750 $\mu\text{g}\cdot\text{mL}^{-1}$) [44], and it is comparable to that of *Cestrum nocturnum* hexane and chloroform extracts (1000 $\mu\text{g}\cdot\text{mL}^{-1}$) [89]. As for *S. sclerotiorum*, higher activities have only been reported for *A. maritima* (375 $\mu\text{g}\cdot\text{mL}^{-1}$) [44] and for some *C. nocturnum* extracts (250–1000 $\mu\text{g}\cdot\text{mL}^{-1}$) [89], and a comparable activity has been reported for *Zingiber officinale* Roscoe essential oil (1000 $\mu\text{g}\cdot\text{mL}^{-1}$) [100].

4.4. Comparison with Synthetic Fungicides

A comparison of the activity of three conventional synthetic fungicides against the three fungal taxa is presented in Table 7. In the case of *B. cinerea* and *S. sclerotiorum*, the

same isolates were used, while a different *F. oxysporum* isolate was used. A comparison with five clinical-grade antibiotics against *P. cichorii* (strain CITA Pci-5) is summarized in Table S2.

With respect to the antifungal activity, the MIC values for *E. serrata* extract and the COS-*E. serrata* conjugate complex (1000 and 500–750 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively) are lower than those of fosetyl-Al. Fosetyl-Al achieved full inhibition of *B. cinerea* and *F. oxysporum* at 2000 $\mu\text{g}\cdot\text{mL}^{-1}$, but it did not fully inhibit *S. sclerotiorum* at this concentration. Furthermore, the MIC values for *E. serrata* extract and the COS-*E. serrata* conjugate complex are much lower than those of azoxystrobin, which did not fully inhibit any of the fungal taxa at 62,500 $\mu\text{g}\cdot\text{mL}^{-1}$. However, the natural-based product would be less effective than mancozeb, for which full inhibition was achieved at 150 $\mu\text{g}\cdot\text{mL}^{-1}$ in all cases.

Regarding the antibacterial activity, *E. serrata* extract and the COS-*E. serrata* conjugate complex achieved full inhibition of *P. cichorii* strain CITA Pci-5 at 750 and 187.5 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table 3). Therefore, at least the latter would be more active than ampicillin to which some *Pseudomonas* spp. are resistant due to their production of β -lactamases.

Table 7. Radial growth of mycelium of *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum* in in vitro experiments using a PDA medium modified with varying concentrations (the recommended dose, 1/10th of the recommended dose, and 10 times the recommended dose) of three synthetic fungicides.

Commercial Fungicide	Pathogen	Mycelium Radial Growth (mm)			Inhibition (%)		Ref.
		Control	Rd/10	Rd *	Rd/10	Rd *	
Azoxystrobin	<i>B. cinerea</i>	75.0	12	51	84	32	[74]
	<i>F. oxysporum</i> spp.	75.0	45.0	40.0	40.0	46.7	[44]
	<i>S. sclerotiorum</i>	75.0	14.0	9.0	81.3	88.0	
Mancozeb	<i>B. cinerea</i>	75.0	0	0	100	100	[74]
	<i>F. oxysporum</i> spp.	75.0	0.0	0.0	100.0	100.0	[44]
	<i>S. sclerotiorum</i>	75.0	0.0	0.0	100.0	100.0	
Fosetyl-Al	<i>B. cinerea</i>	75.0	38	0	49.3	100	[74]
	<i>F. oxysporum</i> spp.	75.0	66.7	0.0	11.1	100.0	[44]
	<i>S. sclerotiorum</i>	75.0	75.0	13.3	0.0	82.2	

* Rd stands for the recommended dose, i.e., 62.5 $\text{mg}\cdot\text{mL}^{-1}$ of azoxystrobin (250 $\text{g}\cdot\text{L}^{-1}$ for Ortiva[®], azoxystrobin 25%), 1.5 $\text{mg}\cdot\text{mL}^{-1}$ of mancozeb (2 $\text{g}\cdot\text{L}^{-1}$ for Vondozeb[®], mancozeb 75%), and 2 $\text{mg}\cdot\text{mL}^{-1}$ of fosetyl-Al (2.5 $\text{g}\cdot\text{L}^{-1}$ for Fosbel[®], fosetyl-Al 80%). The radial growth of the mycelium for the control (PDA) was 75 mm. All mycelial growth values (in mm) are average values ($n = 3$).

5. Conclusions

GC-MS characterization of the *E. serrata* hydromethanolic extract showed that the main constituents were 3-methylbutyl formate (15.5%), quinic acid (11.8%), N1-(4-hydroxybutyl)-N3-methylguanidine acetate (5.3%), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (or DDMP) (4%). With a view to its valorization for borage crop protection, the antimicrobial activity of the extract was tested in vitro against four *P. cichorii* strains and three isolates of *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum*, respectively. Minimum inhibitory concentrations of 750 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ were obtained against the bacteria and the three fungal taxa, respectively. Upon conjugation of the extract with COS, the antimicrobial activity improved, resulting in MIC values of 187.5, 750, 500, and 500 $\mu\text{g}\cdot\text{mL}^{-1}$ for *P. cichorii*, *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum*, respectively. The most active treatment (i.e., COS-*E. serrata* extract conjugate complex) was further tested in in vivo assays against the two pathogens that currently have a higher impact in the main growing areas of borage in Aragón (Spain), confirming a strong protective action at a dose of 375 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ for *P. cichorii* and *F. oxysporum*, respectively. These findings are noteworthy since the plant extract showed higher activity than synthetic fungicides such as azoxystrobin and fosetyl-Al. Therefore, this study calls for further research on the applicability of *E. serrata* extract as a biorational in integrated pest management of borage and other horticultural crops.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae9060652/s1>, Figure S1: *Euphorbia serrata* aerial part and detail of the inflorescences (cyathia); Figure S2: Infrared spectra of *E. serrata* flowers, leaves, and latex; Figure S3: GC–MS chromatogram of *E. serrata* aerial parts hydromethanolic extract; Figure S4: Inhibition of the radial growth of the mycelium of *B. cinerea*, *F. oxysporum*, and *S. sclerotiorum* in the in vitro tests performed with PDA medium amended with different concentrations (in the range of 62.5–1500 $\mu\text{g}\cdot\text{mL}^{-1}$) of chitosan oligomers (COS), *E. serrata* aerial part extract, and their conjugate complex (COS–*E. serrata*). Table S1: Efficacy of plant extracts and essential oils reported in the literature against the phytopathogens under study; Table S2: Minimum inhibitory concentrations (expressed in $\mu\text{g}\cdot\text{mL}^{-1}$) of conventional antibiotics (for clinical use) against *P. cichorii* strain CITA Pci-5.

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