

Antifungal potential of carnosic acid from *Salvia somalensis* against phytopathogenic fungi

Valeria Iobbi¹, Marta Lo Vetere¹, Anna Paola Lanteri², Jakob K. Reinhardt³, Ombeline Danton³, Morris Keller³, Matthias Hamburger³, Annalisa Salis⁴, Gianluca Damonte⁴, Olivier Potterat³, and Angela Bisio^{1*}

¹ Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148 Genova, Italy

² CERSAA Centro di Sperimentazione e Assistenza Agricola, Regione Rollo 98, 17031 Albenga, Italy

³ Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland

⁴ Department of Experimental Medicine, Biochemistry Section, University of Genova, Viale Benedetto XV 1, 16132 Genova, Italy

* Correspondence: angela.bisio@unige.it (A.B.); Tel.: +39-010-335-2637 (A.B.)

Supplementary Materials

Figure S1. ¹H NMR (500 MHz, CD₃OD) spectrum of compound **25**

Figure S2. HSQC (500 MHz, CD₃OD) spectrum of compound **25**

Figure S3. HMBC (500 MHz, CD₃OD) spectrum of compound **25**

Figure S4. COSY (500 MHz, CD₃OD) spectrum of compound **25**

Figure S5. NOESY (500 MHz, CD₃OD) 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 *Salvia somalensis*

Cultivation technique of *S. somalensis*

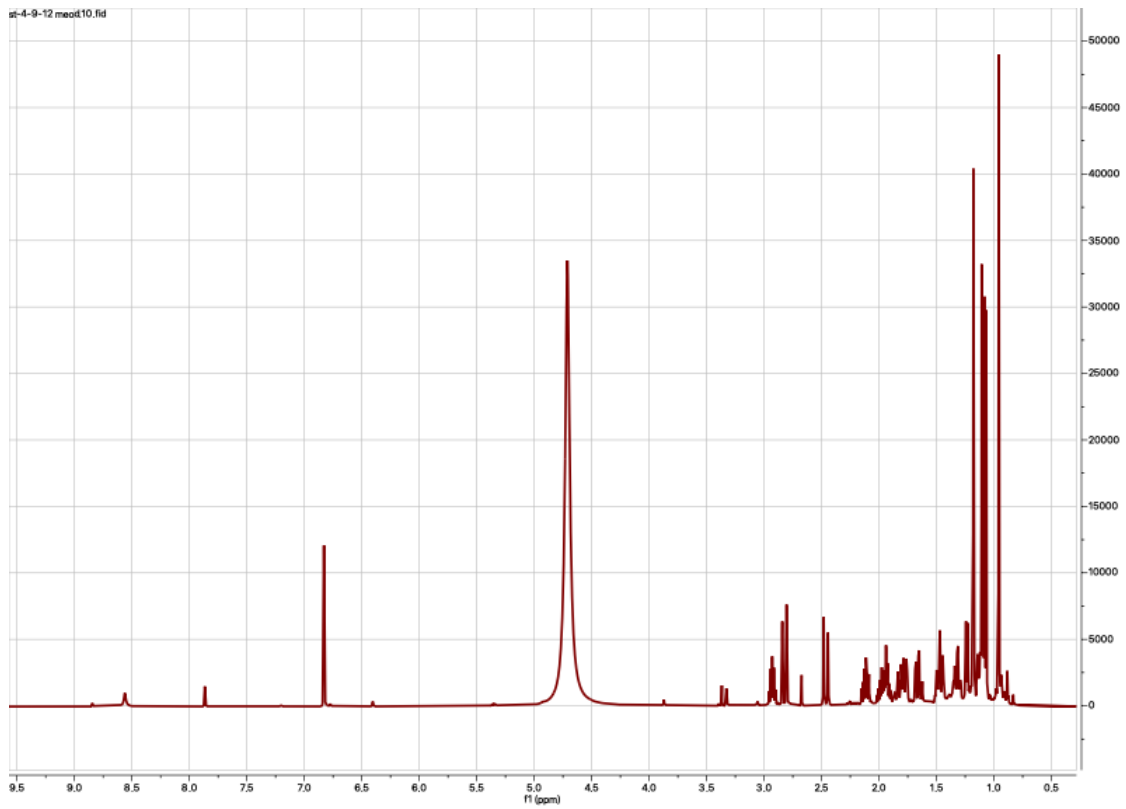


Figure S1. ^1H NMR (500 MHz, CD_3OD) spectrum of compound 25

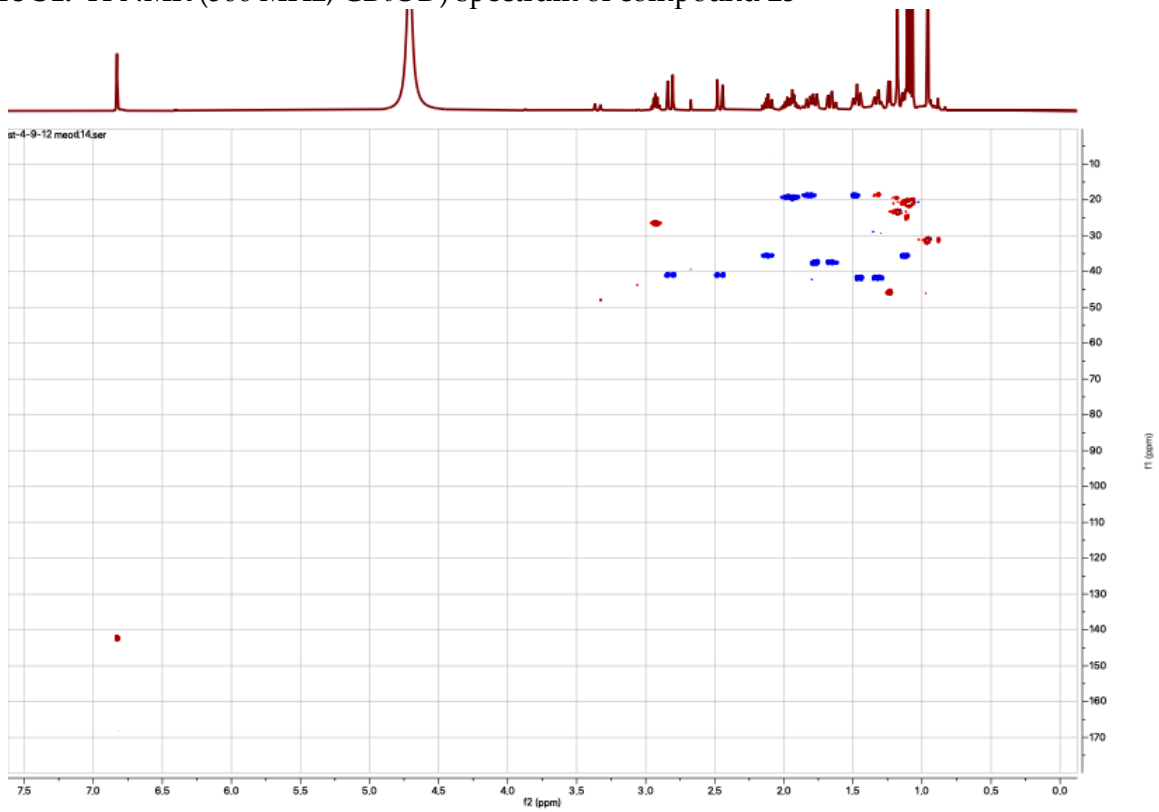


Figure S2. HSQC (500 MHz, CD_3OD) spectrum of compound 25

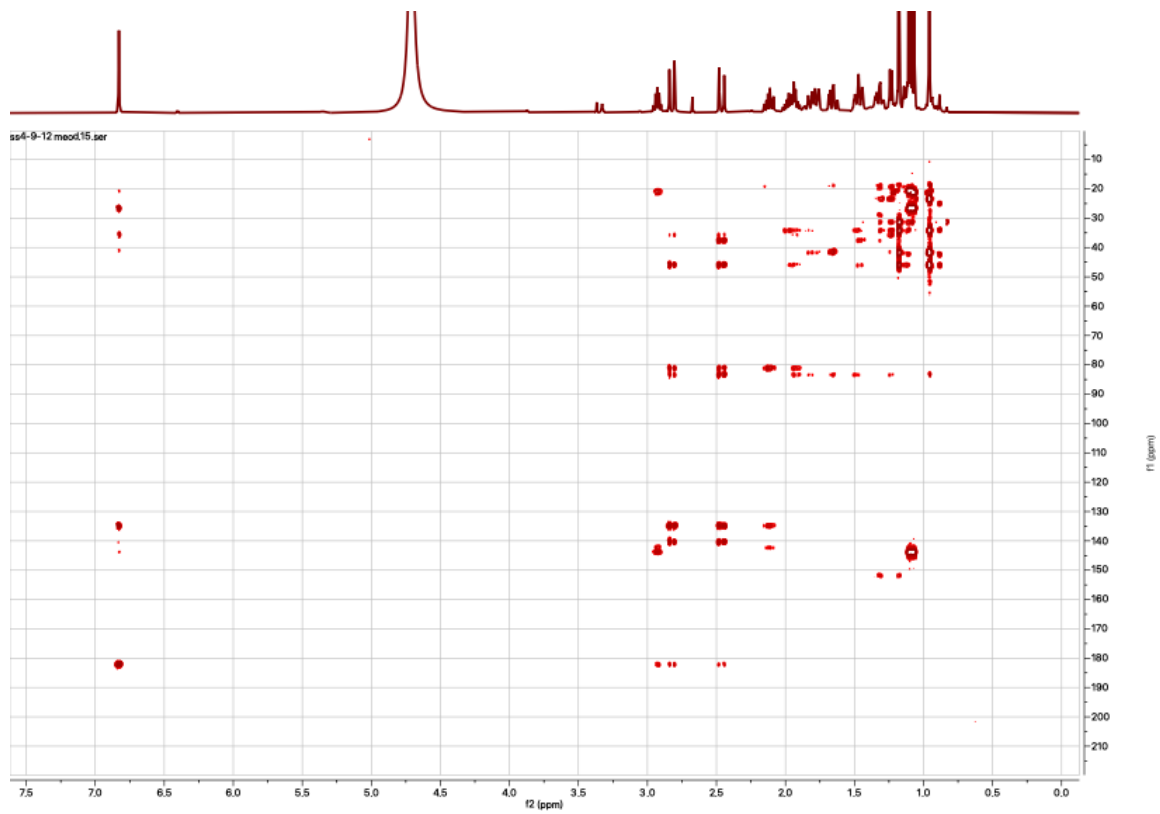


Figure S3. HMBC (500 MHz, CD₃OD) spectrum of compound 25

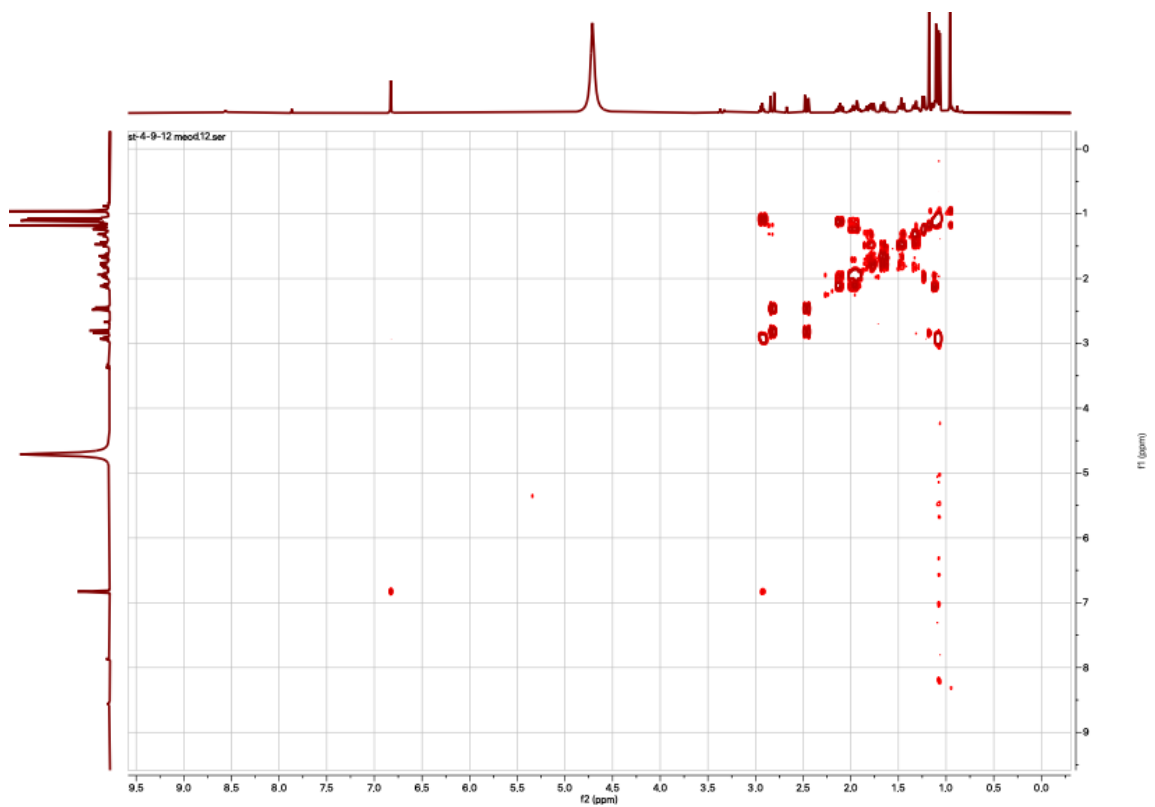


Figure S4. COSY (500 MHz, CD₃OD) spectrum of compound 25

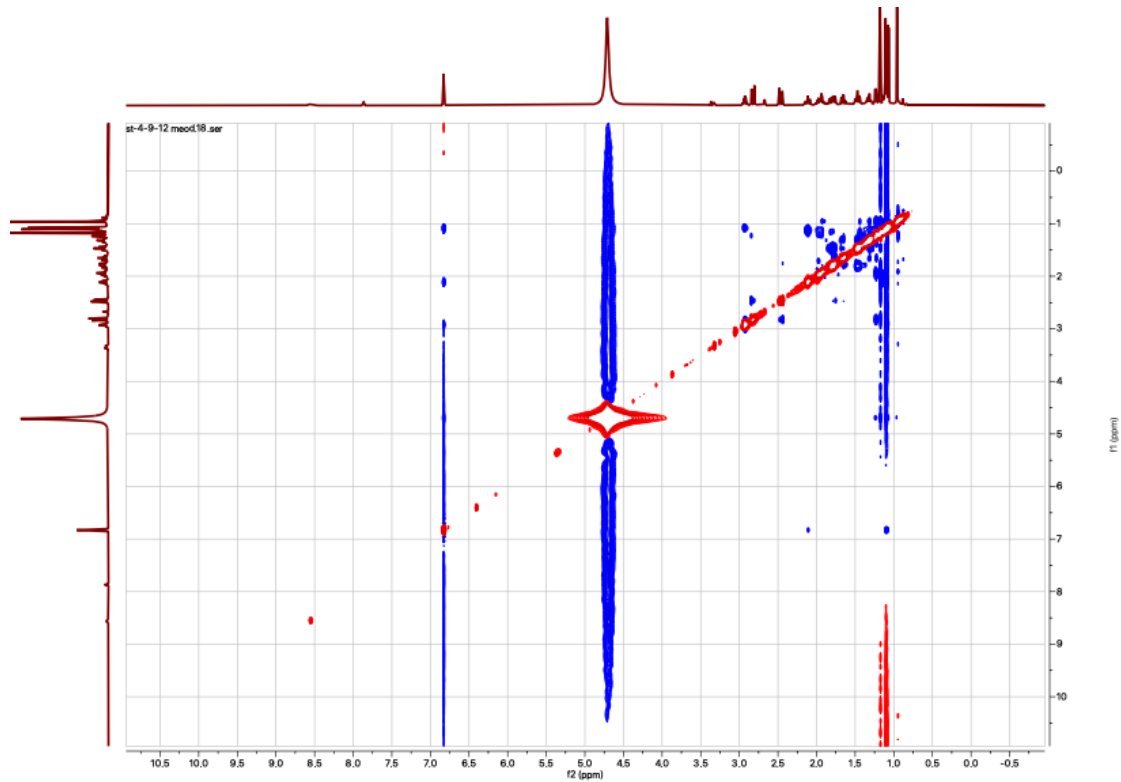


Figure S5. NOESY (500 MHz, CD₃OD) spectrum of compound 25

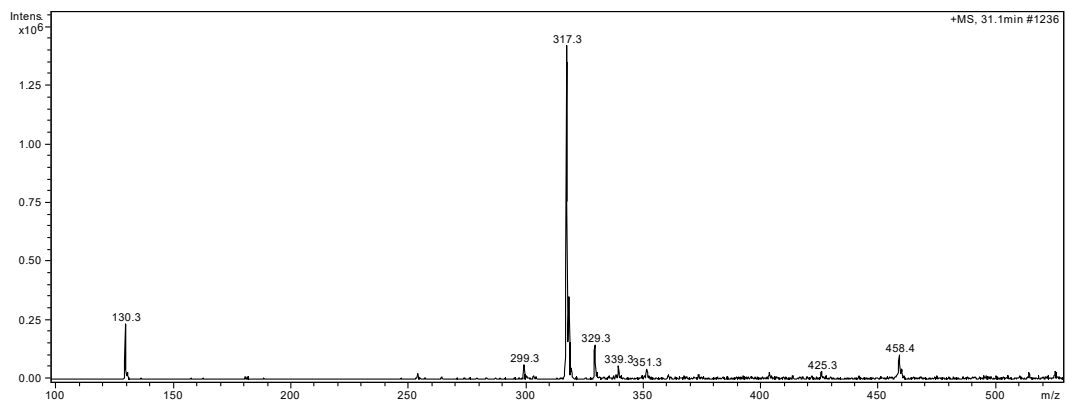


Figure S6. ESIMS spectrum of compound 25

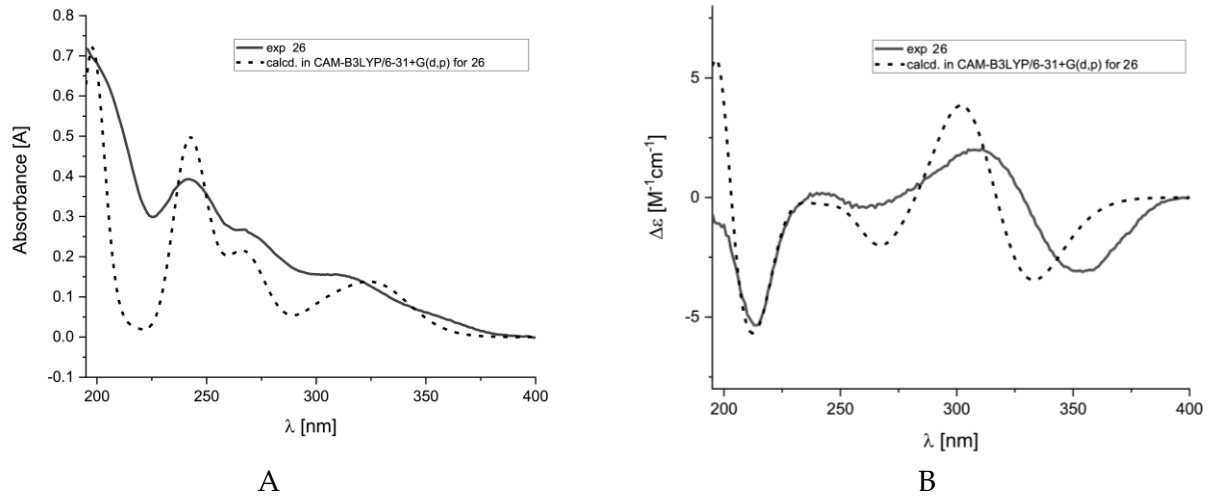


Figure S7. ECD spectra of compound **25**

A: Comparison of the experimental UV spectrum of **25** in MeOH (400 $\mu\text{g/mL}$) to the *ab initio* calculated ECD spectrum of **25**. The calculated spectrum was shifted by +10 nm to align the calculated band with the experimental band at 242 nm. B: Comparison of the experimental ECD spectrum of **25** in MeOH (400 $\mu\text{g/mL}$) to its *ab initio* calculated ECD spectrum. In accordance with the UV data, the calculated spectra were shifted by +10 nm.

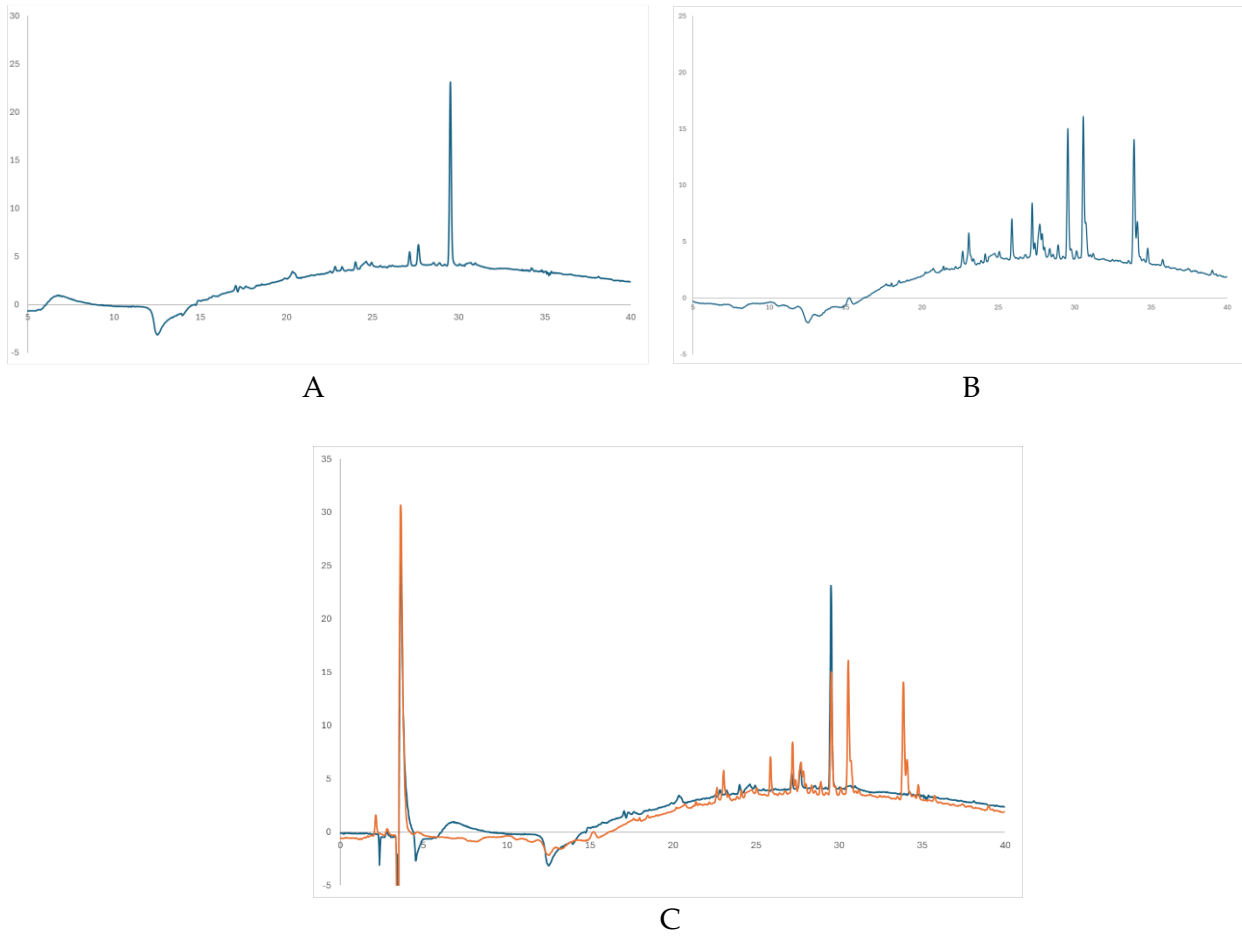


Figure S8. Representative chromatograms of the HPLC profiles of carnosic acid and of the dichloromethane plant surface extract of the fresh aerial parts of *Salvia somalensis*

Representative chromatograms of the HPLC profiles of carnosic acid (A) (t_r 29.0 min), of the dichloromethane plant surface extract of the fresh aerial parts of *S. somalensis* (B) and overlap of both chromatograms (C) at 280 nm.

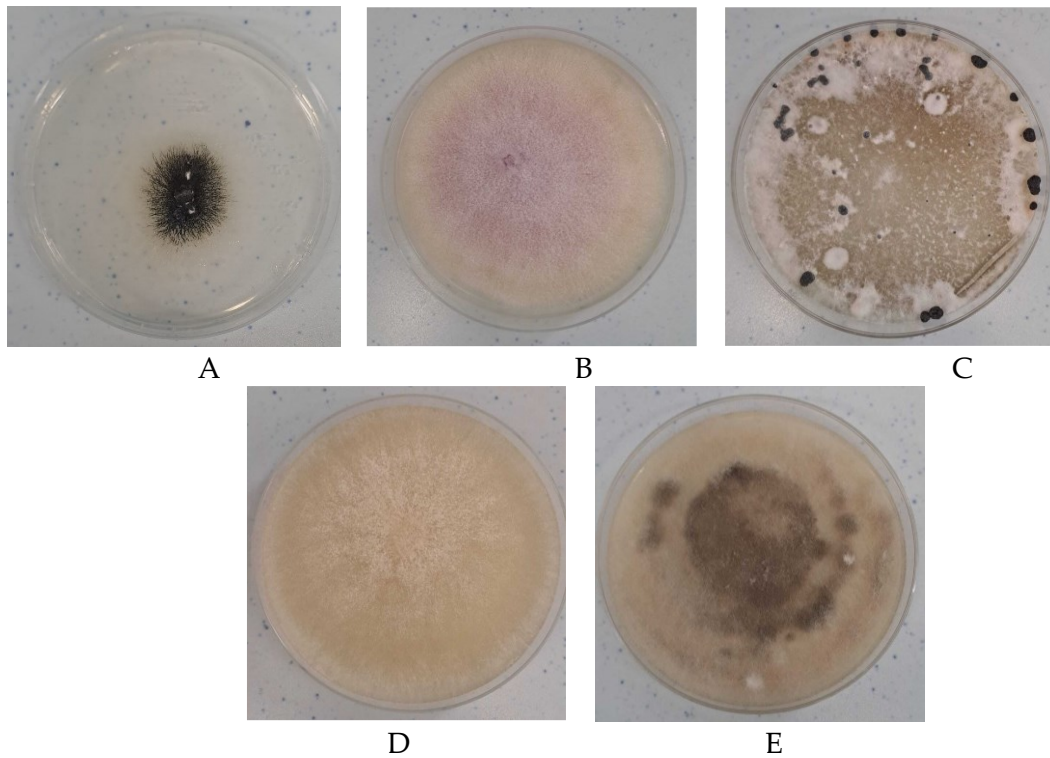


Figure S9. Mycelial growth on potato dextrose agar (PDA) medium of *Colletotrichum coccodes*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Botrytis cinerea* used in the antifungal activity assay

A: *C. coccodes*; B: *F. oxysporum*; C: *S. sclerotiorum*; D: *R. solani*; E: *B. cinerea*

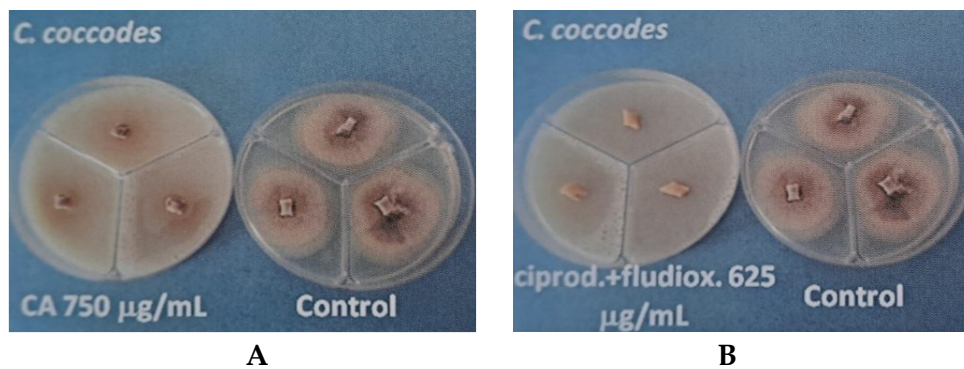


Figure S10. Representative inhibition of growth of fungal mycelium after inoculation on poisoned media

Inhibition of growth of *Colletotrichum coccodes* mycelium 5 days after inoculation on poisoned medium with carnosic acid (CA) at 750 µg/mL (A) compared to the untreated control and to the mycelium treated with cyprodinil + fludioxonil (375+250 µg/mL) (B).

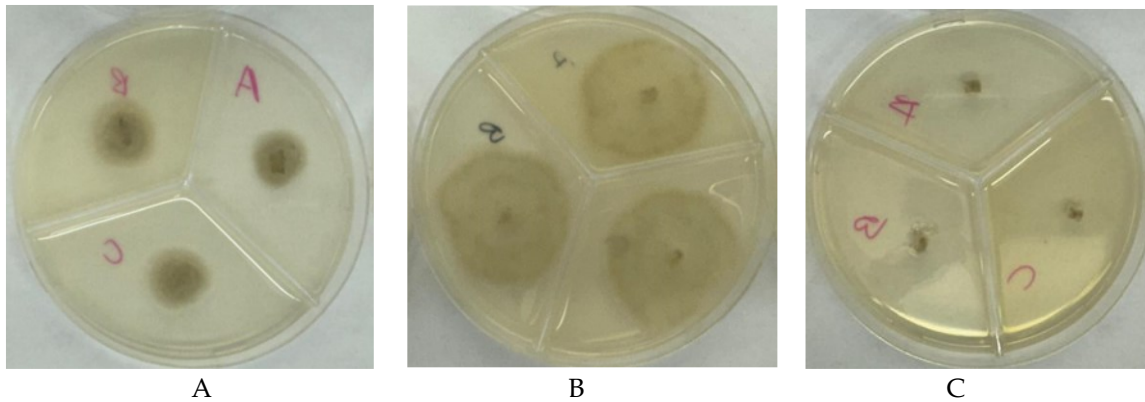


Figure S11. Inhibition of growth of *Botrytis cinerea* (strain 2) mycelium 5 days after inoculation on poisoned media

Inhibition of growth of *B. cinerea* (strain 2) mycelium 5 days after inoculation on poisoned medium with carnosic acid at 1000 µg/mL (A), compared to the untreated control (B) and to the mycelium treated with cyprodinil + fludioxonil (375+250 µg/mL) (C).

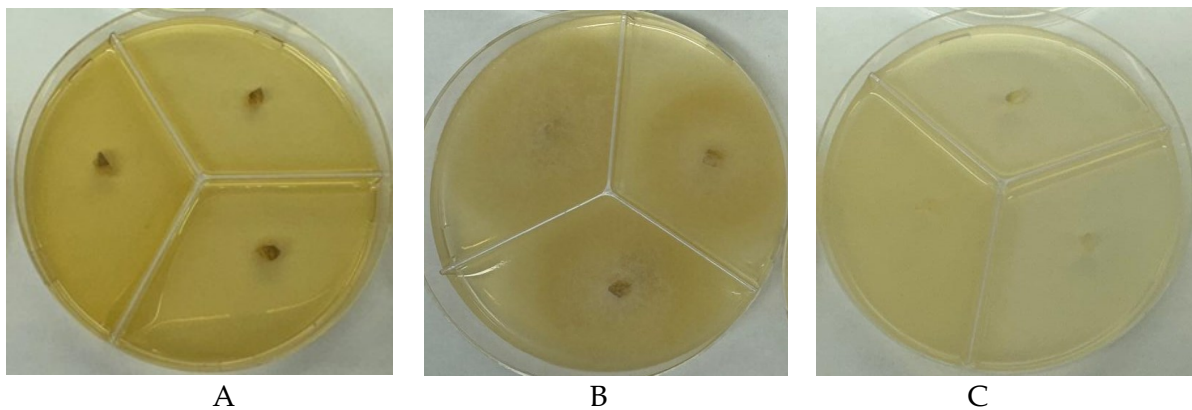
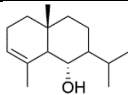
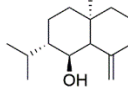
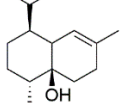
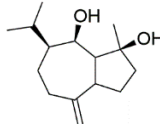
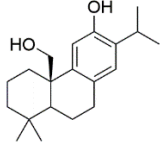
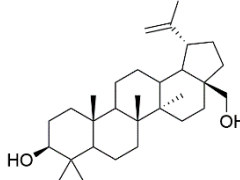
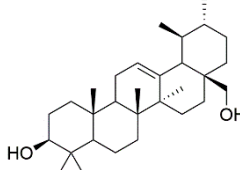
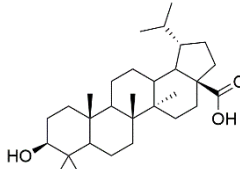
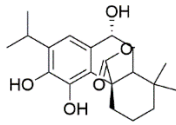
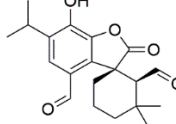
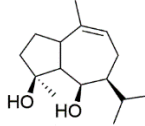


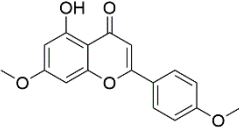
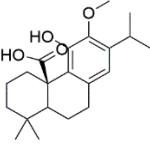
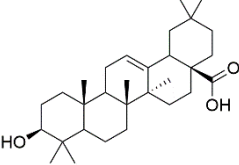
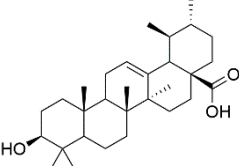
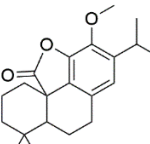
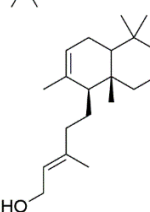
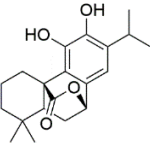
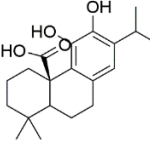
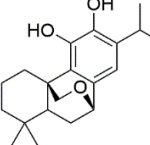
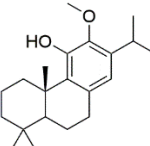
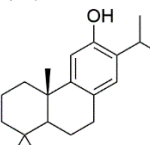
Figure S12. Inhibition of growth of *Botrytis cinerea* (strain 7) mycelium 5 days after inoculation on poisoned media

Inhibition of growth of *B. cinerea* (strain 7) mycelium 5 days after inoculation on poisoned medium with carnosic acid at 5 µg/mL (A) compared to the untreated control (B) and to the mycelium treated with cyprodinil + fludioxonil (375+250 µg/mL) (C).

Table S1. Compounds isolated from the dichloromethane extract of the fresh aerial parts of *Salvia somalensis*

Compound code	Compound name	Chemical structure	References
1	(-)-7- <i>epi</i> -isojunenol		[64]
2	(+)-junenol		[65]
3	(+)- <i>ent</i> -epicubenol		[66]
4	teucladiol		[67]
5	pisiferol		[78, 79]
6	betulin		[73]
7	uvaol		[74]
8	betulinic acid		[73]
9	rosmanol		[80,81]
10	rosmadial		[82]
11	4 β ,6 β -dihydroxy-1 α ,5 α (H)-guai-9-ene		[68]



12	5-hydroxy-7,4'-dimethoxyflavone		[76]
13	12-methylcarnosic acid		[83,84]
14	oleanolic acid		[75]
15	ursolic acid		[75]
16	12-O-methyl-acetylcarnosate		[85]
17	7,13-E-labdadien-15-ol		[77]
18	carnosol		[84]
19	carnosic acid		[84]
20	20-deoxocarnosol		[90]
21	11-hydroxy-12-methoxyabieta-8,11,13-triene		[91]
22	ferruginol		[92]



23	β -chaenocephalol		[69]
24	1 β ,6 β -dihydroxy-4(14)-eudesmene		[70,71]
25	4 α ,9 α -epoxy-2H-dibenzo[a,d]cyclohepten-7(5H)-one		[95]
26	galdosol		[88,89]
27	14-hydroxy-7-O-methylrosmanol		[86]
28	12-methylcarnosol		[87]
29	brussonol		[91,95]
30	demethylsalvicanol		[91]
31	3,11-dihydroxy-3,7,11-trimethyldodeca-1,6,9-triene		[72]
32	11-hydroxysugiol		[93]
33	formosanoxide		[94]



Cultivation technique of *S. somalensis*

Salvia somalensis Vatke, Linnaea 43: 93 (1881)



Group J of *Salvia* in Africa [47]

Salvia s.l. clade I (*Salvia* s.s.), subclade I-A [116]

Distribution: Somalia

There are no works available that report a specific cultivation technique for *S. somalensis*. Considering the characteristics of the species, it is possible to briefly describe the following cultivation practice.

Climate and location: *S. somalensis* prefers a warm, sunny climate.

Soil: this plant prefers well-drained and light soil.

Irrigation: *S. somalensis* does not like excessively humid soil and suffers greatly from waterlogging.

Pruning: to stimulate tillering, it is necessary to proceed with 2-3 prunings during the first 2-3 months of cultivation.

Fertilization: Since this is a shrubby species, it is necessary to guarantee a balanced supply of nutrients (N:P₂O₅:K₂O = 1:1:1).

Multiplication: *S. somalensis* can be propagated by cuttings or by dividing the tufts. Cuttings root easily in well-drained soil, maintaining constant moisture until they develop roots.

Growing density: *S. somalensis* can be grown at a density of 4-6 plants/m².

Diseases and parasites: There are no reported pathogens and parasites attacking *S. somalensis*. It is occasionally possible to detect the presence of whiteflies on young plants.