

Activity of anthracenediones and flavoring phenols in hydromethanolic extracts of *Rubia tinctorum* against grapevine phytopathogenic fungi

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SUPPORTING INFORMATION

Table S1. Repetitions for each of the plant/treatment combinations in the greenhouse bioassay. Each grafted plant was inoculated at two sites below grafting point.

Plant	Treatment	Pathogen	Number of replicates
'Tempranillo' (CL. 32 clone) on 775P rootstock	COS- <i>R. tinctorum</i>	<i>D. seriata</i>	5
		<i>D. viticola</i>	5
		<i>N. parvum</i>	5
	None (negative control)	<i>D. seriata</i>	3
		<i>D. viticola</i>	4
		<i>N. parvum</i>	4
'Garnacha' (VCR3 clone) on 110R rootstock	COS- <i>R. tinctorum</i>	<i>D. seriata</i>	5
		<i>D. viticola</i>	5
		<i>N. parvum</i>	5
	None (positive control)	<i>D. seriata</i>	3
		<i>D. viticola</i>	4
		<i>N. parvum</i>	4

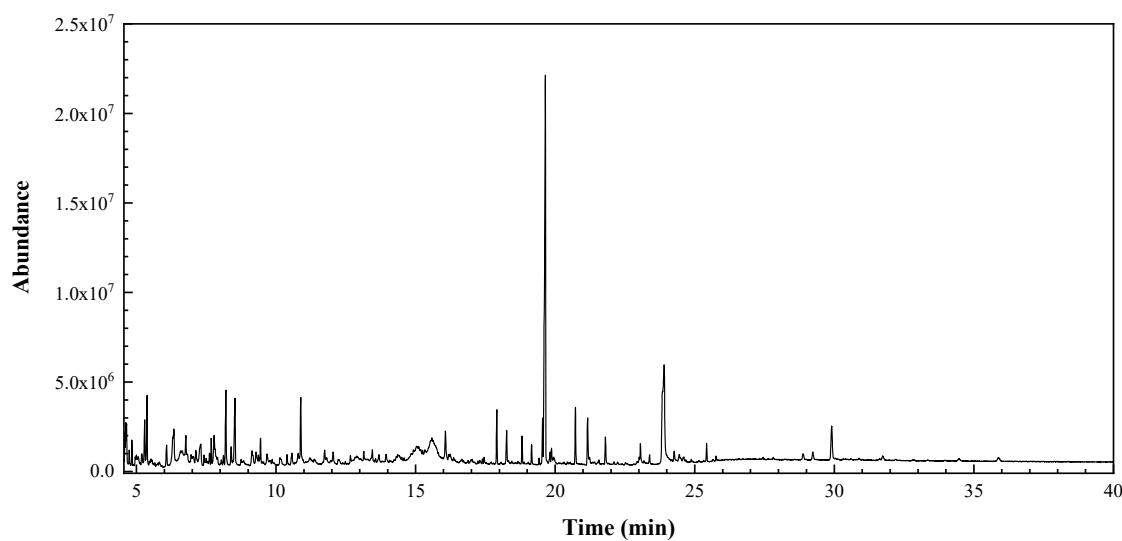


Figure S1. GC-MS spectrum of *R. tinctorum* root hydromethanolic extract

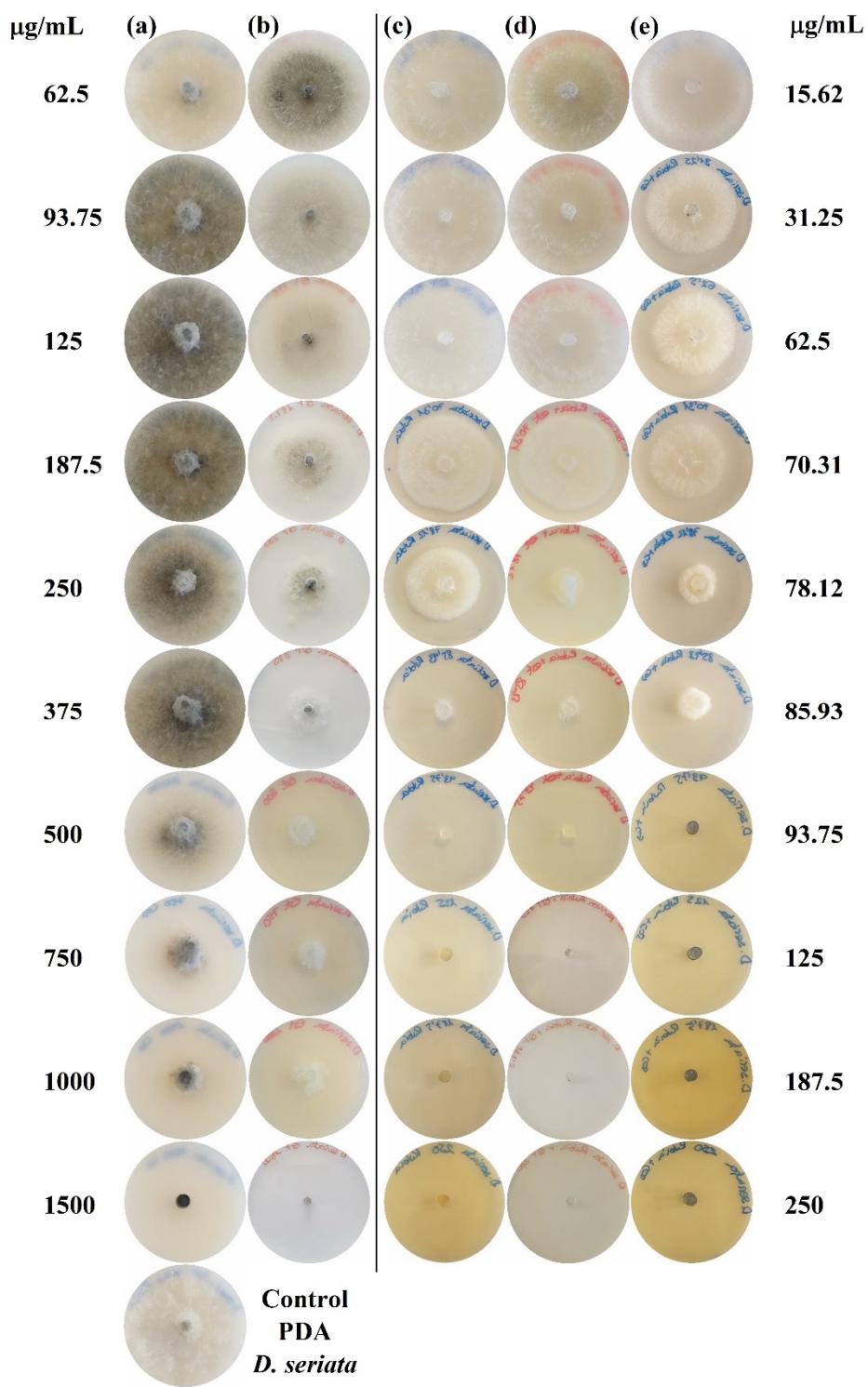


Figure S2. Mycelial growth inhibition of *D. seriata* upon treatment with: (a) chitosan oligomers, COS; (b) stevioside; (c) *R. tinctorum* hydromethanolic extract; (d) stevioside–*R. tinctorum* conjugate complex; (e) COS–*R. tinctorum* conjugate complex at different concentrations. Only one replicate is shown.

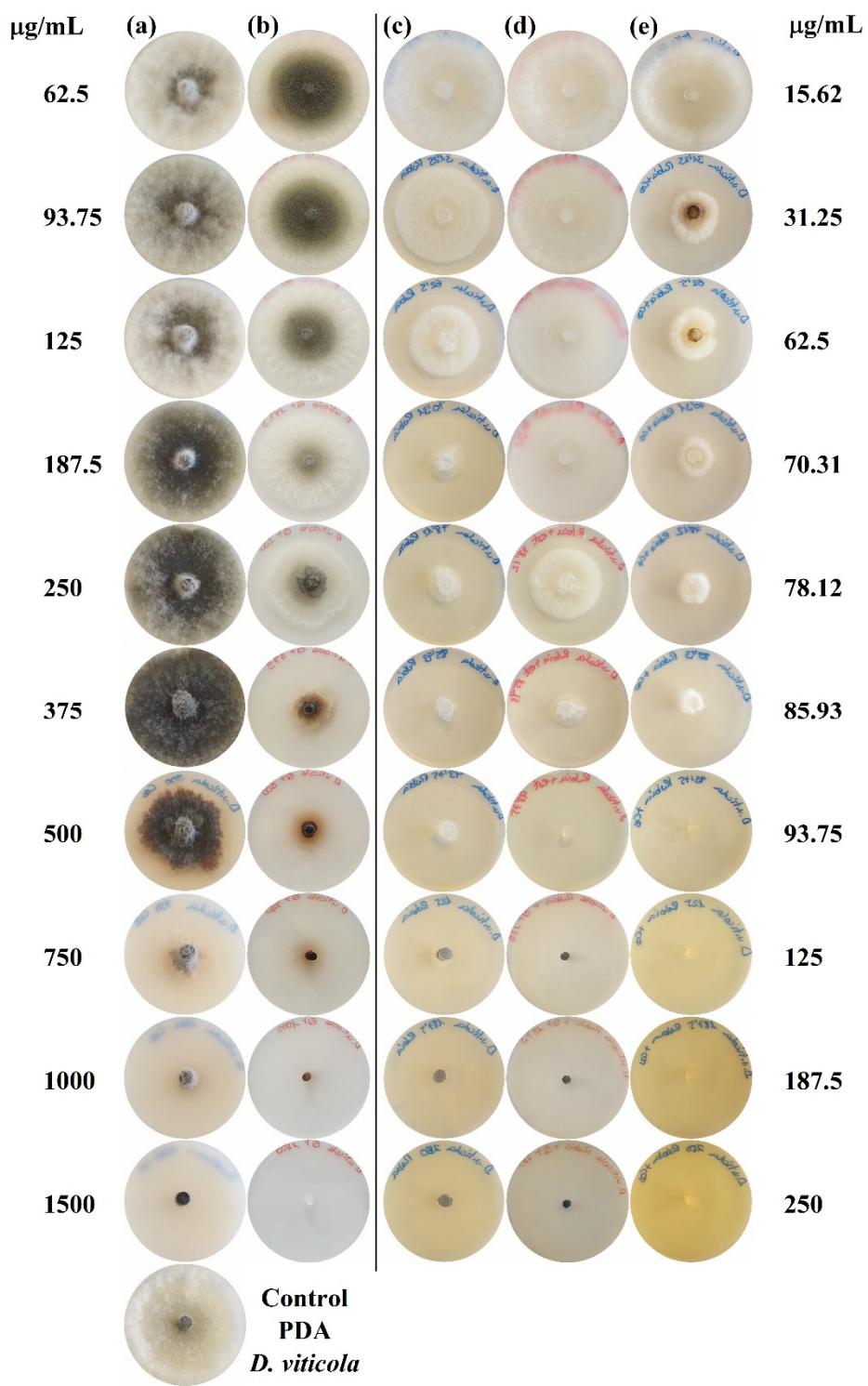


Figure S3. Mycelial growth inhibition of *D. viticola* upon treatment with: (a) chitosan oligomers, COS; (b) stevioside; (c) *R. tinctorum* hydromethanolic extract; (d) stevioside–*R. tinctorum* conjugate complex; (e) COS–*R. tinctorum* conjugate complex at different concentrations. Only one replicate is shown.

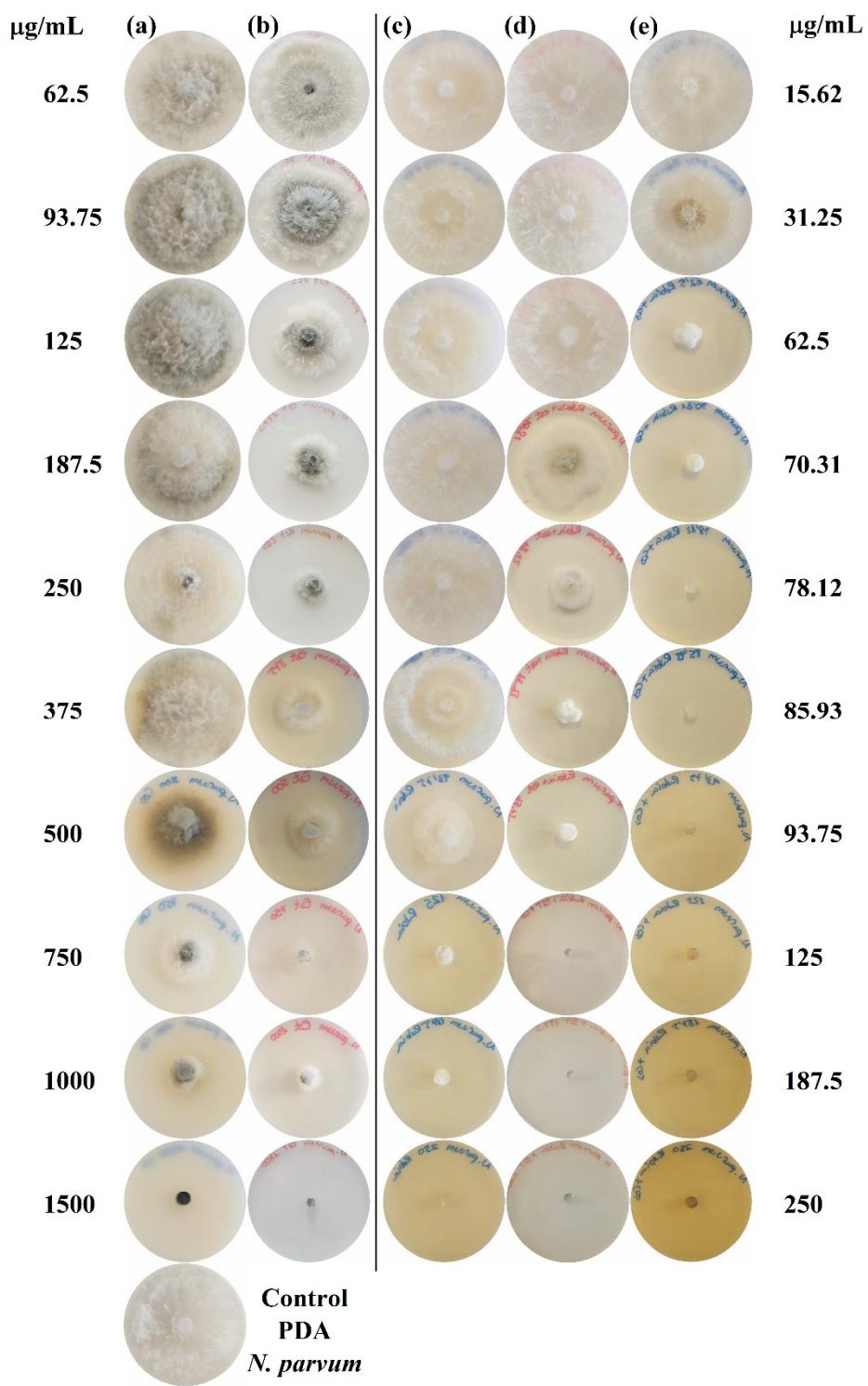


Figure S4. Mycelial growth inhibition of *N. parvum* upon treatment with: (a) chitosan oligomers, COS; (b) stevioside; (c) *R. tinctorum* hydromethanolic extract; (d) stevioside–*R. tinctorum* conjugate complex; (e) COS–*R. tinctorum* conjugate complex, at different concentrations. Only one replicate is shown.

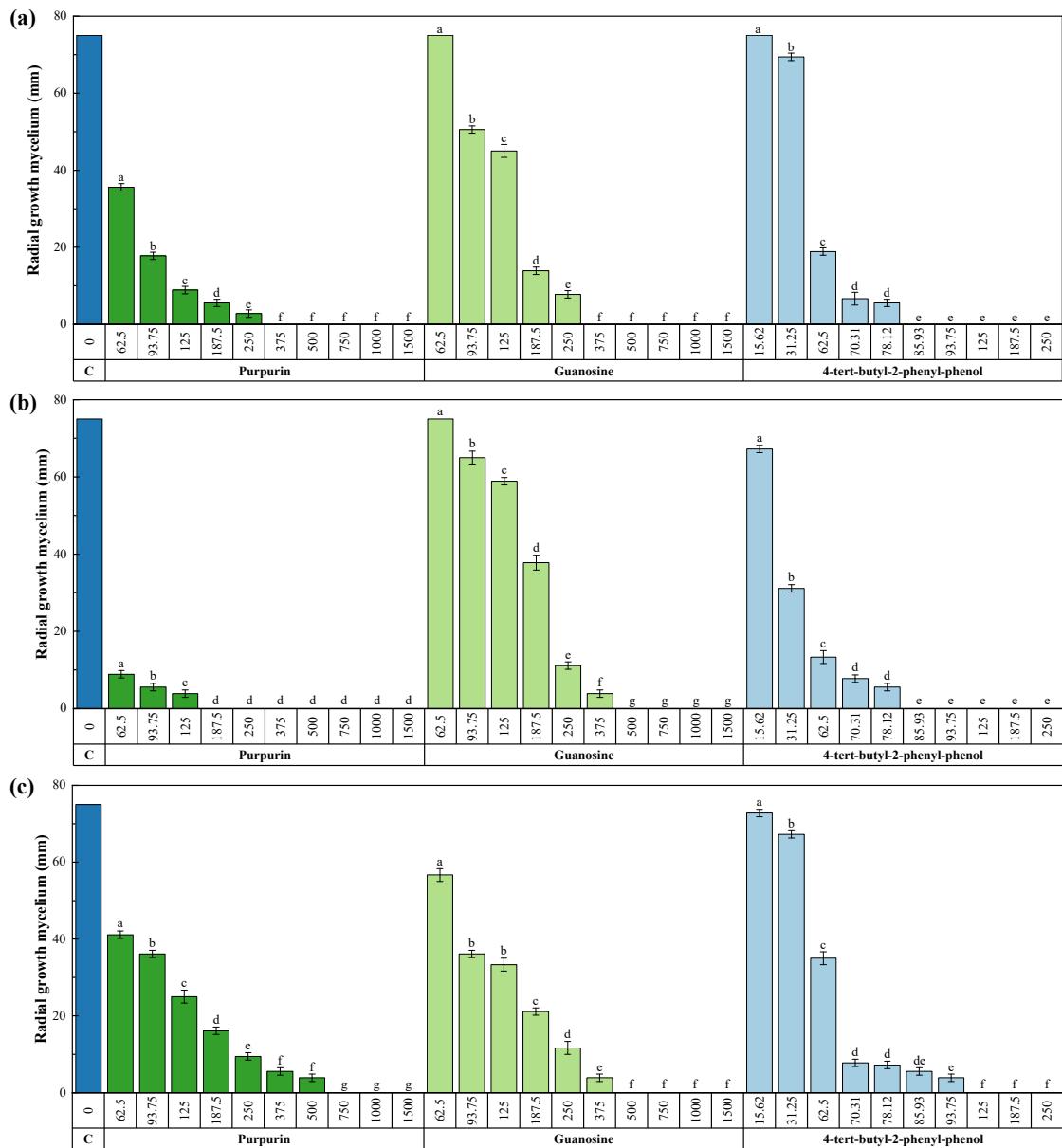


Figure S5. Colony growth measures of (a) *D. seriata*, (b) *D. viticola* and (c) *N. parvum* strains when cultured in PDA plates containing the main phytochemicals found in *R. tinctorum* hydromethanolic extracts (viz. purpurin, guanosine and 4-tert-butyl-2-phenyl-phenol) at concentrations in the 62.5–1500 and 15.62–250 $\mu\text{g}\cdot\text{mL}^{-1}$ range for the least and the most active products, respectively. The same letters above concentrations indicate that they are not significantly different at $p < 0.05$. Error bars represent standard deviations.

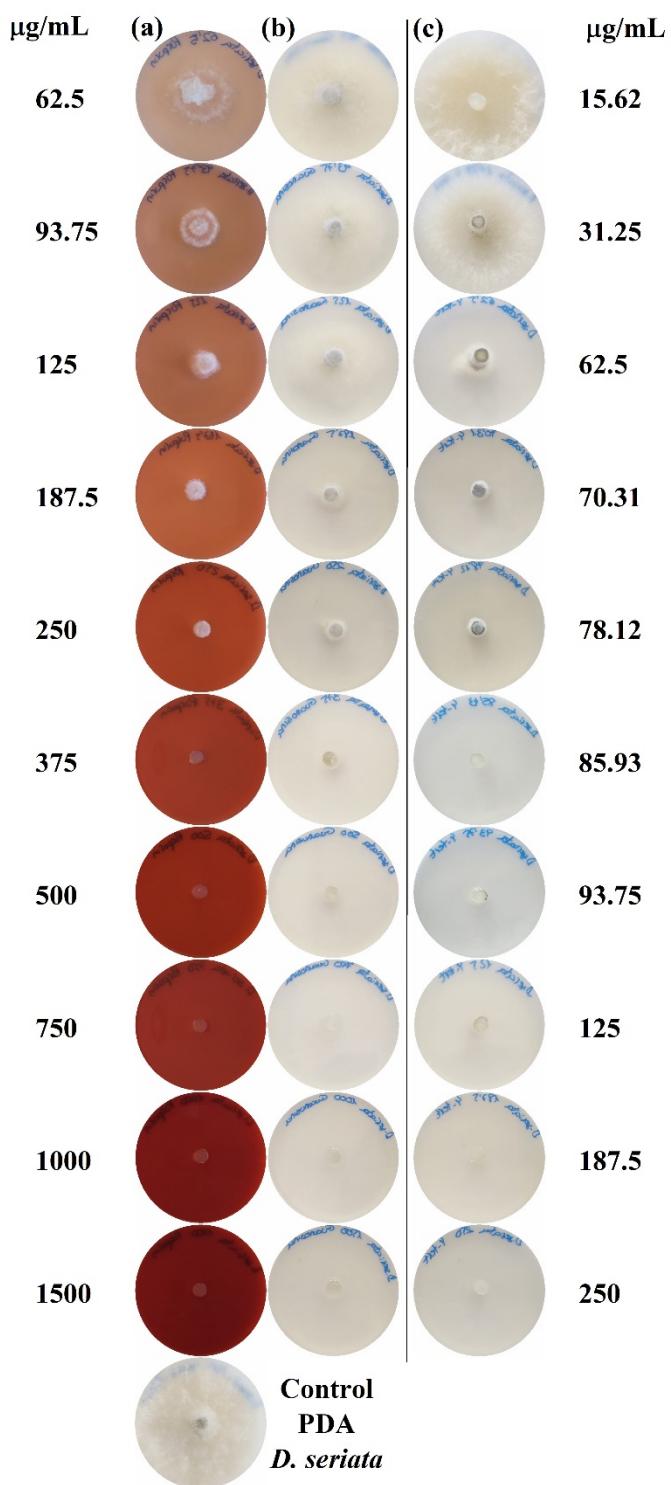


Figure S6. Mycelial growth inhibition of *D. seriata* upon treatment with the main phytochemicals found in *R. tinctorum* hydromethanolic extracts: (a) purpurin, (b) guanosine, and (c) 4-tert-butyl-2-phenyl-phenol, at different concentrations. Only one replicate is shown.

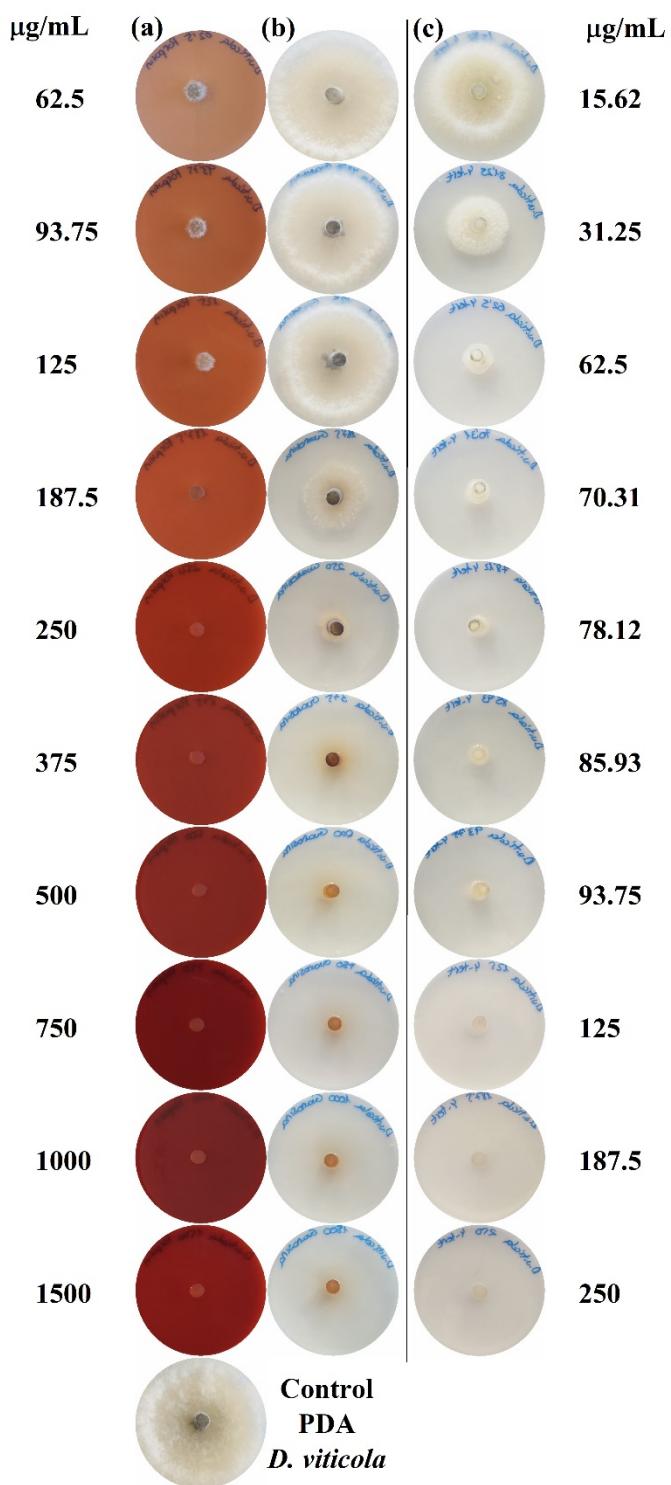


Figure S7. Mycelial growth inhibition of *D. viticola* upon treatment with the main phytochemicals found in *R. tinctorum* hydromethanolic extracts: (a) purpurin, (b) guanosine, and (c) 4-tert-butyl-2-phenyl-phenol, at different concentrations. Only one replicate is shown.

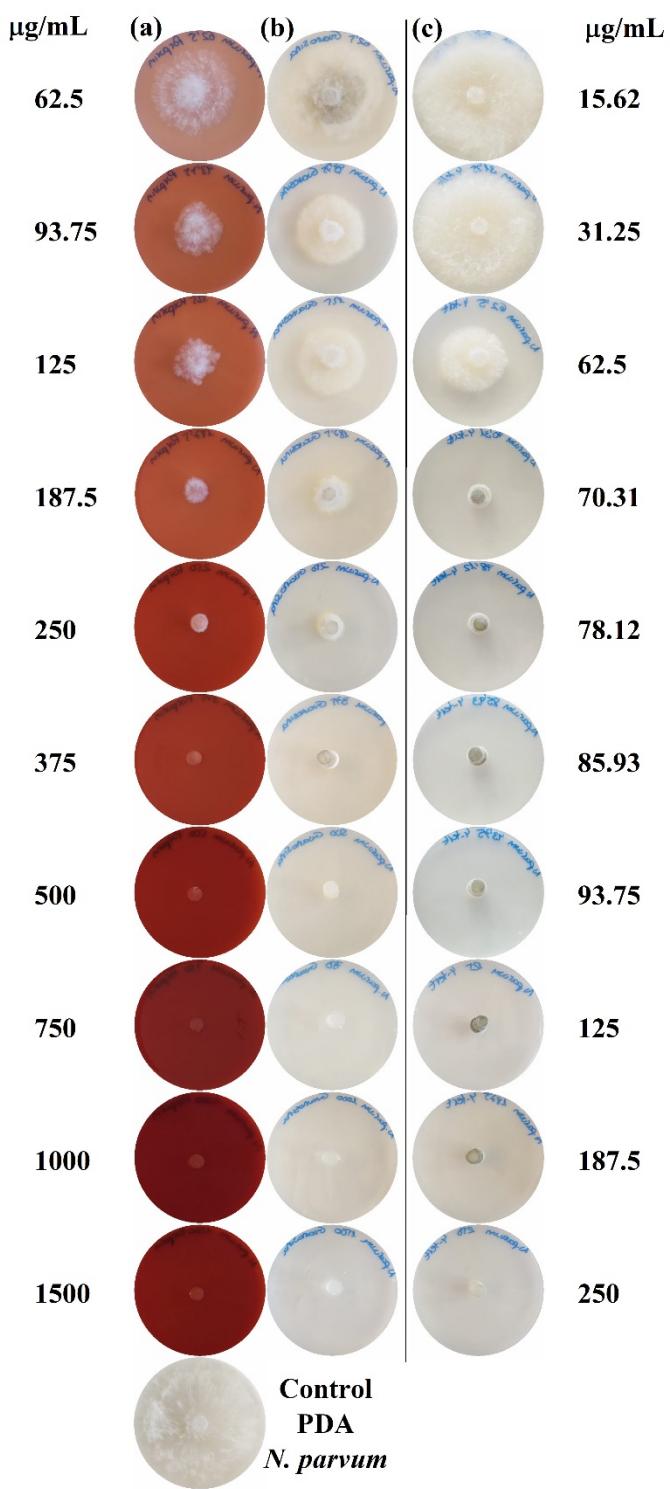


Figure S8. Mycelial growth inhibition of *N. parvum* upon treatment with the main phytochemicals found in *R. tinctorum* hydromethanolic extracts: (a) purpurin, (b) guanosine, and (c) 4-tert-butyl-2-phenyl-phenol, at different concentrations. Only one replicate is shown.

Table S2. Examples of application of *R. tinctorum* extracts against microorganisms reported in the literature.

Pathogen	Assayed product	Effectiveness	Ref.
<i>Antibacterial activity</i>			
<i>Shigella dysenteriae</i>	Aqueous extract	MIC = 25-100 mg·mL ⁻¹	
<i>Aeromonas hydrophila</i>	Methanol extract	MIC = 5-50 mg·mL ⁻¹	
<i>Escherichia coli</i>			[1]
<i>Klebsiella</i> spp.			
<i>Serratia marcescens</i>			
<i>Staphylococcus aureus</i>			
<i>Bacillus cereus</i> RSKK 863	Aqueous solution of commercial natural dye powder (10 g/300 mL)	Inhibition zone (mm)	
<i>Bacillus megaterium</i> RSKK 5117		-	
<i>Shigella sonnei</i> RSKK 877		8.5	
<i>Staphylococcus aureus</i> ATCC 25923		-	
<i>Bacillus subtilis</i> RSKK 244		9.7	[2]
<i>Pseudomonas aeruginosa</i> ATCC 29212		-	
<i>Salmonella</i> sp. 21.3		10.2	
<i>P. aeruginosa</i> ATCC 27853		-	
<i>Streptococcus epidermidis</i>		-	
<i>Staphylococcus aureus</i> ATCC 25923	Methanolic extract, without / with acid hydrolysis at 1 mg·mL ⁻¹	Inhibition diameter (mm)	
<i>Listeria monocytogenes</i> ATCC 11120		17.0±0.7 / 19.4±0.5	
<i>Pseudomonas aeruginosa</i> ATCC 9027		19.3±0.9 / 21.1±0.7	
<i>Salmonella enteritidis</i> ATCC 14028		14.7±0.3 / 16.6±0.4	[3]
<i>Escherichia coli</i> ATCC 25922		12.2±0.7 / 15.5±0.0	
<i>Aeromonas hydrophila</i> ATCC 1943		10.1±0.3 / 11.2±0.6	
<i>Klebsiella pneumoniae</i> ATCC 13833		13.4±0.8 / 14.3±0.1	
<i>Staphylococcus aureus</i>	Methanolic extract	MIC = 0.1562 mg·mL ⁻¹	
<i>Bacillus subtilis</i>		MIC = 0.1562 mg·mL ⁻¹	
<i>Escherichia coli</i>		MIC = 0.3125 mg·mL ⁻¹	[4]
<i>Pseudomonas aeruginosa</i>		MIC = 0.3125 mg·mL ⁻¹	
<i>Escherichia coli</i> ATCC 11230	Ethanol, methanol, ethyl acetate and water extracts (5 mg/mL)	+	
<i>Enterobacter aerogenes</i> ATCC 13048		+	
<i>Proteus vulgaris</i> ATCC 8427		-	
<i>Serratia marcescens</i> NRRL 3284		-	
<i>Bacillus cereus</i> ATCC 7064		+	
<i>Bacillus subtilis</i> ATCC 6633		+	[5]
<i>Micrococcus luteus</i> LA 2971		+	
<i>Staphylococcus aureus</i> ATCC 6538P		+	
<i>Klebsiella pneumoniae</i> UC57		-	
<i>Pseudomonas aeruginosa</i> ATCC 27853		+	
<i>Bacillus subtilis</i>	aqueous / methanolic extracts	+	
<i>Bacillus cereus</i>	(50 g/100 mL)	+	[6]
<i>Bacillus mycoides</i>		+	
<i>Escherichia coli</i> ATCC 25922	Aqueous / ethanolic extracts	MIC = 500 / 1000 µg·mL ⁻¹	[7]
<i>Staphylococcus aureus</i> ATCC 6538	Silk dyed with madder	+	
<i>Escherichia coli</i> ATCC 25922		-	[8]
<i>Staphylococcus aureus</i>	PET fabric dyed with madder	86% inhibition	
<i>Escherichia coli</i>			[9]
<i>Antifungal activity</i>			
<i>Candida albicans</i> DSMZ 1386	Silk dyed with madder	None	[10]
<i>Aspergillus niger</i>	Methanolic extract, with and without acid hydrolysis	Inhibition diameter	
<i>Aspergillus ochraceus</i>	(1 mg/mL)	19.6±0.8 / 21.4±0.8 mm	[3]
<i>Candida albicans</i> ATCC 10231	Ethanol, methanol, ethyl acetate and water extracts (5 mg/mL)	17.9±0.1 / 19.6±0.4 mm	
<i>Saccharomyces cerevisiae</i> ATCC 9763			
<i>Geotrichum penicillatum</i>	for yeasts, 10-100 µg/mL for filamentous fungi)	-	[5]
<i>Aspergillus flavus</i>		Full inhibition at 100 µg·mL ⁻¹	
<i>Fusarium oxysporum</i>		Full inhibition at 100 µg·mL ⁻¹	
<i>Candida</i> spp.	Purpurin	MIC = 1.28–5.12 µg·mL ⁻¹	[11]

	<i>R. tinctorum</i> roots methanolic extract / alizarin (concentration not reported)	% inhibition
<i>Trichoderma viride</i>	43 / 50	[12]
<i>Doratomyces stemonitis</i>	41 / 20	
<i>Aspergillus niger</i>	22 / 20	
<i>Penicillium verrucosum</i>	35 / 22	
<i>Alternaria alternata</i>	18 / 18	
<i>Aueobasidium pullulans</i>	18 / 14	
<i>Mucor mucedo</i>	22 / 31	
<i>Penicillium expansum</i>	aqueous / methanolic extracts	+ / +
<i>Aspergillus niger</i>		+ / +
<i>Alternaria alternata</i>		+ / +
<i>Geotrichum candidum</i>		+ / +
<i>Fusarium solani</i>		+ / +
<i>Postia placenta</i>	Wood treated with aqueous extract	+
<i>Trametes versicolor</i>	(1:20 mass plant material to liquid)	+
Actinomycetes		[13]
<i>Streptomyces murinus</i> ISP 5091	Ethanol, methanol, ethyl acetate and water extracts (10-100 µg/mL)	Full inhibition at 100 µg·mL ⁻¹ [5]

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