

## Supplementary Material

# **Trend of Antioxidant Activity and Total Phenolic Content in Wild Pabular Plants as Part of the Environmental Quality Assessment of Some Areas in the Central Italy**

Ivan Notardonato <sup>1</sup>, Francesca Fantasma <sup>2</sup>, Pamela Monaco <sup>2</sup>, Cristina Di Fiore <sup>1</sup>, Gabriella Saviano <sup>2</sup>, Carmen Giancola <sup>3</sup>, Pasquale Avino <sup>1,\*</sup> and Vincenzo De Felice<sup>2</sup>

<sup>1</sup> Department of Agriculture, Environmental and Food Sciences, University of Molise, Via De Sanctis, I-86100 Campobasso, Italy

<sup>2</sup> Department of Biosciences and Territory, University of Molise, C. da Fonte Lappone, I-86090 Pesche, Italy

<sup>3</sup> Consorzio del Giardino della Flora Appenninica, I-86062 Capracotta, Italy

\* Correspondence: avino@unimol.it; Tel.: +39-0874-404634

**Table S1.** Total phenol content in wild pabular plants harvested in the four study areas investigated during the three sampling periods. Results are expressed as mg GAE g<sup>-1</sup> DM  $\pm$  SD, where DM is dry matter and SD is standard deviation; n.a. means that the sample was not available.

Plant Species	Sampling I	Sampling II	Sampling III
<b>MONTEFORTE</b>			
<i>Trifolium pratense</i>	9.27 $\pm$ 2.62	n.a.	5.23 $\pm$ 0.08
<i>Scorzonera laciniata</i>	2.73 $\pm$ 0.11	n.a.	n.a.
<i>Bromus erectus</i>	7.09 $\pm$ 0.74	2.84 $\pm$ 0.07	2.30 $\pm$ 0.03
<i>Centaurea ambigua</i>	n.a.	1.57 $\pm$ 0.13	n.a.
<i>Festuca circummediterranea</i>	n.a.	3.52 $\pm$ 0.18	0.76 $\pm$ 0.05
<b>VERRINO</b>			
<i>Medicago lupulina</i>	2.84 $\pm$ 0.08	n.a.	n.a.
<i>Lotus corniculatus</i>	6.74 $\pm$ 1.21	n.a.	n.a.
<i>Thymus longicaulis</i>	14.37 $\pm$ 0.35	17.03 $\pm$ 0.30	4.32 $\pm$ 0.10
<i>Festuca circummediterranea</i>	n.a.	3.98 $\pm$ 0.08	1.02 $\pm$ 0.01
<i>Dorycnium pentaphyllum</i>	n.a.	2.79 $\pm$ 0.22	n.a.
<i>Trifolium pratense</i>	n.a.	n.a.	5.30 $\pm$ 0.20
<b>GUARDATA</b>			
<i>Trifolium pratense</i>	7.92 $\pm$ 0.64	7.45 $\pm$ 0.42	5.06 $\pm$ 0.37
<i>Festuca circummediterranea</i>	3.26 $\pm$ 0.52	2.01 $\pm$ 0.01	0.99 $\pm$ 0.05
<i>Dactylis glomerata</i>	3.49 $\pm$ 1.15	7.59 $\pm$ 0.21	2.93 $\pm$ 0.06
<b>GUADO CANNAVINA</b>			
<i>Hippocrepis comosa</i>	9.75 $\pm$ 0.23	4.15 $\pm$ 0.04	1.29 $\pm$ 0.12
<i>Festuca circummediterranea</i>	2.80 $\pm$ 0.21	3.33 $\pm$ 0.23	1.24 $\pm$ 0.06
<i>Dactylis glomerata</i>	4.83 $\pm$ 0.78	7.82 $\pm$ 0.44	2.93 $\pm$ 0.33

**Table S2.** Total tannin content in wild pabular plants harvested in the four study areas investigated during the three sampling periods. Results are expressed as mg TAE g<sup>-1</sup> DM± SD, where DM is dry matter and SD is standard deviation; n.a. means that the sample was not available.

Plant Species	Sampling I	Sampling II	Sampling III
<b>MONTEFORTE</b>			
<i>Trifolium pratense</i>	10.62±0.08	n.a.	5.11±0.04
<i>Scorzonera laciniata</i>	2.68±0.13	n.a.	n.a.
<i>Bromus erectus</i>	6.97±0.5	2.68±0.23	1.98±0.05
<i>Centaurea ambigua</i>	n.a.	1.41±0.35	n.a.
<i>Festuca circummediterranea</i>	n.a.	3.14±0.23	0.55±0.05
<b>VERRINO</b>			
<i>Medicago lupulina</i>	2.81±0.14	n.a.	n.a.
<i>Lotus corniculatus</i>	5.63±0.38	n.a.	n.a.
<i>Thymus longicaulis</i>	14.09±0.15	15.50±0.05	2.93±0.06
<i>Festuca circummediterranea</i>	n.a.	3.69±0.17	0.68±0.05
<i>Dorycnium pentaphyllum</i>	n.a.	2.77±0.37	n.a.
<i>Trifolium pratense</i>	n.a.	n.a.	3.99±0.23
<b>GUARDATA</b>			
<i>Trifolium pratense</i>	8.10±0.60	5.57±0.31	4.09±0.21
<i>Festuca circummediterranea</i>	2.80±0.32	1.84±0.05	0.80±0.03
<i>Dactylis glomerata</i>	2.61±0.31	6.77±0.27	1.95±0.47
<b>GUADO CANNAVINA</b>			
<i>Hippocrepis comosa</i>	9.12±0.24	3.87±0.41	0.78±0.13
<i>Festuca circummediterranea</i>	2.55±0.07	2.86±0.58	0.99±0.23
<i>Dactylis glomerata</i>	4.13±0.19	6.64±0.34	2.06±0.14

**Table S3.** Condensed tannins content in wild pabular plants harvested in the four study areas investigated during the three sampling periods. Results are expressed as  $\mu\text{g mL}^{-1}$  of extract  $\pm$  standard deviation (SD); n.a. means that the sample was not available.

Plant Species	Sampling I	Sampling II	Sampling III
<b>MONTEFORTE</b>			
<i>Trifolium pratense</i>	3.59 $\pm$ 0.14	n.a.	10.51 $\pm$ 0.08
<i>Scorzonera laciniata</i>	1.23 $\pm$ 0.08	n.a.	n.a.
<i>Bromus erectus</i>	1.92 $\pm$ 0.22	0.13 $\pm$ 0.02	1.49 $\pm$ 0.03
<i>Centaurea ambigua</i>	n.a.	0.46 $\pm$ 0.03	n.a.
<i>Festuca circummediterranea</i>	n.a.	0.67 $\pm$ 0.13	1.74 $\pm$ 0.08
<b>VERRINO</b>			
<i>Medicago lupulina</i>	2.44 $\pm$ 0.17	n.a.	n.a.
<i>Lotus corniculatus</i>	6.03 $\pm$ 0.05	n.a.	n.a.
<i>Thymus longicaulis</i>	0.85 $\pm$ 0.08	0.77 $\pm$ 0.07	1.33 $\pm$ 0.12
<i>Festuca circummediterranea</i>	n.a.	0.63 $\pm$ 0.22	1.97 $\pm$ 0.18
<i>Dorycnium pentaphyllum</i>	n.a.	22.85 $\pm$ 0.10	n.a.
<i>Trifolium pratense</i>	n.a.	n.a.	2.82 $\pm$ 0.07
<b>GUARDATA</b>			
<i>Trifolium pratense</i>	1.56 $\pm$ 0.06	3.33 $\pm$ 0.27	2.49 $\pm$ 0.31
<i>Festuca circummediterranea</i>	2.49 $\pm$ 0.12	0.77 $\pm$ 0.08	1.67 $\pm$ 0.32
<i>Dactylis glomerata</i>	3.44 $\pm$ 0.14	2.03 $\pm$ 0.25	1.95 $\pm$ 0.08
<b>GUADO CANNAVINA</b>			
<i>Hippocrepis comosa</i>	0.95 $\pm$ 0.13	3.87 $\pm$ 0.33	1.64 $\pm$ 0.05
<i>Festuca circummediterranea</i>	0.67 $\pm$ 0.08	1.23 $\pm$ 0.17	1.21 $\pm$ 0.34
<i>Dactylis glomerata</i>	2.49 $\pm$ 0.22	3.46 $\pm$ 0.43	1.87 $\pm$ 0.08

**Table S4.** Antioxidant activity of wild pabular plants investigated in the four study areas investigated during the three sampling periods. Results are expressed as IC<sub>50</sub> (mg mL<sup>-1</sup>) with related standard deviation (DS); n.a. means that the sample was not available.

Plant Species	Sampling I	Sampling II	Sampling III
<b>MONTEFORTE</b>			
<i>Trifolium pratense</i>	1.73±0.28	n.a.	1.02±0.09
<i>Scorzonera laciniata</i>	2.43±0.75	n.a.	n.a.
<i>Bromus erectus</i>	2.39±0.64	3.76±0.08	2.12±0.01
<i>Centaurea ambigua</i>	n.a.	8.76±0.13	n.a.
<i>Festuca circummediterranea</i>	n.a.	2.76±0.16	3.26±0.34
<b>VERRINO</b>			
<i>Medicago lupulina</i>	9.83±1.13	n.a.	n.a.
<i>Lotus corniculatus</i>	3.02±0.93	n.a.	n.a.
<i>Thymus longicaulis</i>	1.02±0.02	0.50±0.01	0.49±0.01
<i>Festuca circummediterranea</i>	n.a.	3.92±0.76	2.84±0.11
<i>Dorycnium pentaphyllum</i>	n.a.	4.24±0.28	n.a.
<i>Trifolium pratense</i>	n.a.	n.a.	0.31±0.01
<b>GUARDATA</b>			
<i>Trifolium pratense</i>	3.18±0.87	0.89±0.30	0.41±0.01
<i>Festuca circummediterranea</i>	7.76±0.13	6.08±0.26	3.36±0.06
<i>Dactylis glomerata</i>	7.69±2.68	0.95±0.09	0.79±0.03
<b>GUADO CANNAVINA</b>			
<i>Hippocrepis comosa</i>	5.01±1.24	6.23±0.15	5.93±0.72
<i>Festuca circummediterranea</i>	8.39±0.67	4.60±0.11	2.60±0.05
<i>Dactylis glomerata</i>	3.25±0.28	1.35±0.14	0.84±0.09

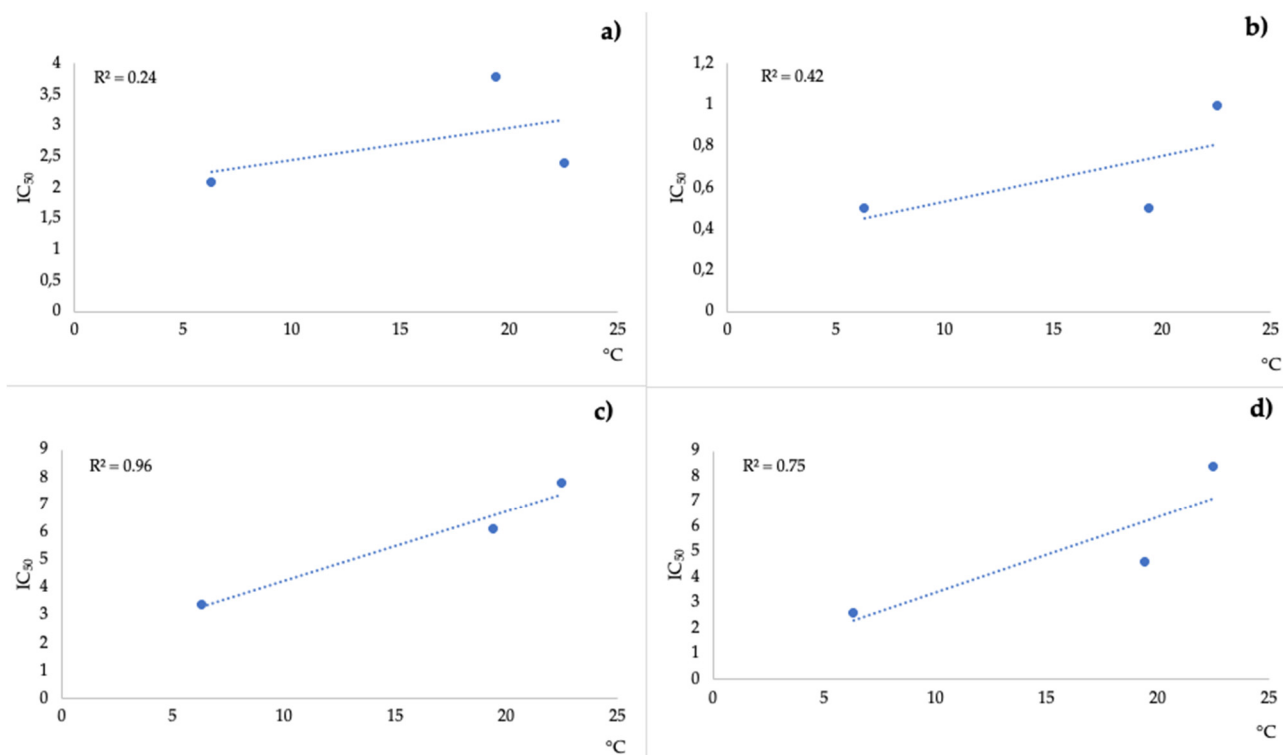
**Table S5.** Heavy metals concentrations in wild pabular plants analysed expressed as  $\mu\text{g g}^{-1}$  with standard deviation. Cd, Ni and Pb were below LODs values.

	Location	Plant species	Cu	Zn
Sampling I	Monteforte	<i>T. pratense</i>	10.7±1.1	1.1±0.1
		<i>S. laciniata</i>	8.1±1.0	1.4±0.1
		<i>B. erectus</i>	4.6±0.2	3.5±0.3
	Verrino	<i>M. lupulina</i>	2.3±0.3	6.2±0.8
		<i>L. corniculatus</i>	3.1±0.3	5.2±0.5
		<i>T. longicaulis</i>	3.0±0.4	8.3±1.1
	Guardata	<i>T. pratense</i>	6.0±0.9	3.3±0.3
		<i>F. circummediterranea</i>	1.2±0.2	5.8±0.5
		<i>D. glomerata</i>	2.0±0.3	3.1±0.3
	Guado Cannavina	<i>H. comosa</i>	5.5±0.6	4.1±0.7
		<i>F. circummediterranea</i>	2.3±0.1	2.4±0.2
		<i>D. glomerata</i>	3.4±0.5	5.0±0.6
Sampling II	Monteforte	<i>B. erectus</i>	1.5±0.3	7.6±0.8
		<i>C. ambigua</i>	15.5±0.3	16.2±2.3
		<i>F. circummediterranea</i>	1.3±0.2	20.9±2.6
	Verrino	<i>T. longicaulis</i>	16.1±0.5	16.5±2.1
		<i>F. circummediterranea</i>	12.9±1.0	9.4±1.5
		<i>D. pentaphyllum</i>	18.1±0.6	19.5±2.9
	Guardata	<i>T. pratense</i>	13.3±1.0	18.8±2.7
		<i>D. glomerata</i>	3.1±0.3	17.3±1.4
		<i>F. circummediterranea</i>	1.4±0.2	12.1±1.1
	Guado Cannavina	<i>D. glomerata</i>	20.4±1.1	23.1±0.6
		<i>F. circummediterranea</i>	12.0±0.6	15.2±1.9
		<i>H. comosa</i>	15.9±0.9	17.8±1.0
Sampling III	Monteforte	<i>T. pratense</i>	19.2±1.4	12.0±1.1
		<i>B. erectus</i>	12.3±1.2	5.6±0.7
		<i>F. circummediterranea</i>	16.3±0.5	6.7±0.7
	Verrino	<i>T. pratense</i>	20.1±0.9	11.1±1.7
		<i>T. longicaulis</i>	18.0±0.6	16.3±1.3
		<i>F. circummediterranea</i>	11.0±0.3	13.3±2.0
	Guardata	<i>T. pratense</i>	20.1±1.3	19.8±3.1
		<i>F. circummediterranea</i>	13.5±0.6	24.5±3.9
		<i>D. glomerata</i>	16.5±0.7	17.4±2.7
	Guado Cannavina	<i>H. comosa</i>	13.1±0.9	26.5±2.3
		<i>D. glomerata</i>	15.1±0.9	20.5±2.7
		<i>F. circummediterranea</i>	14.3±1.3	19.3±2.2

**Table S6.** Heavy metals concentrations in soil samples of areas analysed expressed as  $\mu\text{g g}^{-1}$  with standard deviation. Pb and Cd were below the LOD values.

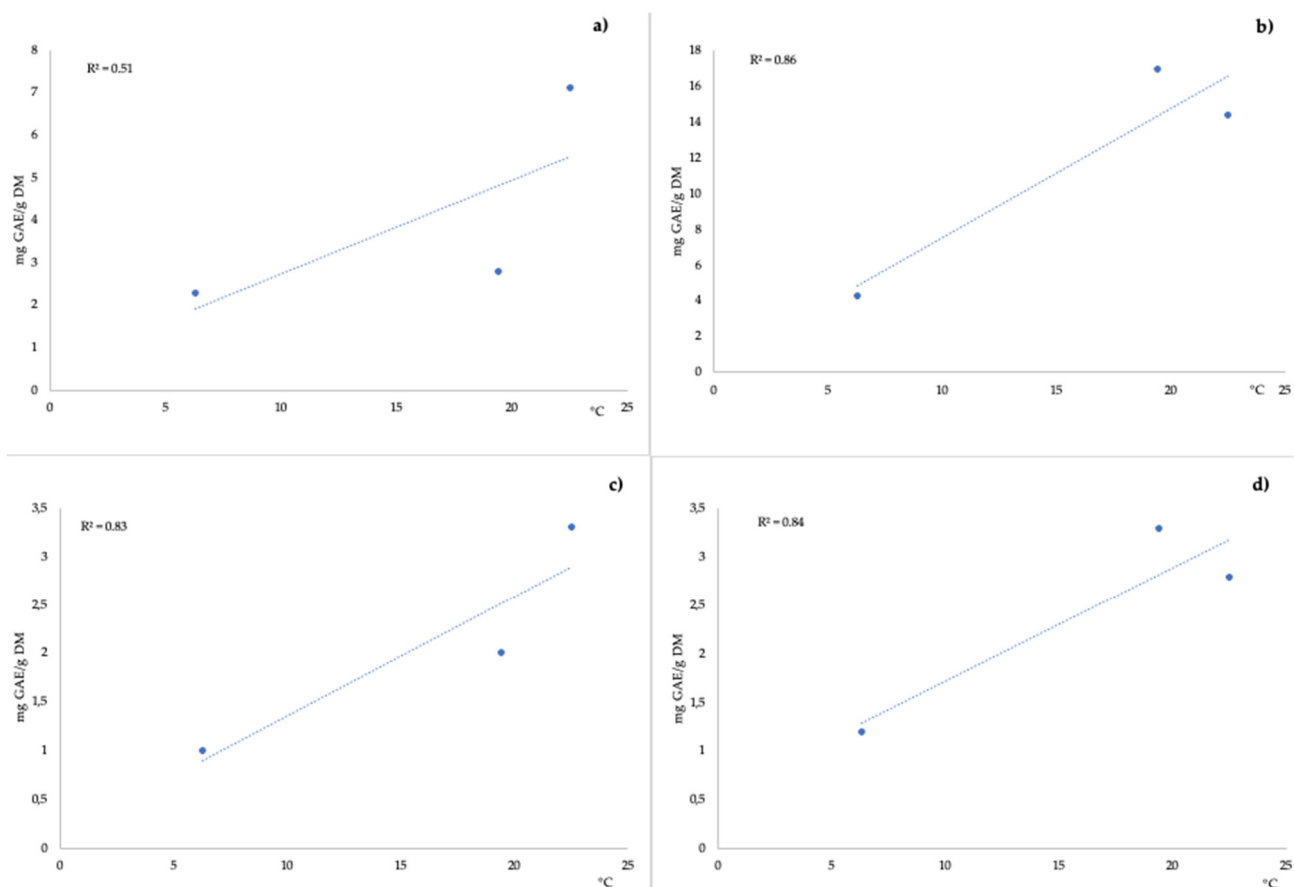
Area	Cu	Ni	Zn
Monteforte	59.6 $\pm$ 0.8	36.3 $\pm$ 4.9	54.7 $\pm$ 5.3
Verrino	26.2 $\pm$ 0.9	13.2 $\pm$ 0.8	40.2 $\pm$ 2.5
Guardata	21.1 $\pm$ 0.3	14.5 $\pm$ 2.2	47.8 $\pm$ 7.4
Guado Cannavina	24.5 $\pm$ 1.6	24.0 $\pm$ 1.4	38.6 $\pm$ 5.8

**Figure S1.** Correlation between the antioxidant activity and the sampling period, considering the mean temperatures of each sampling time. Please note: the correlation has been evaluated only when plant species was present during all period of sampling. **a)** *B. erectus* collected from Monteforte; **b)** *T. longicaulis* collected from Verrino; **c)** *F. circummediterranea* collected from Guardata; **d)** *F. circummediterranea* collected from Guado Cannavina; For correlation value: see the text.

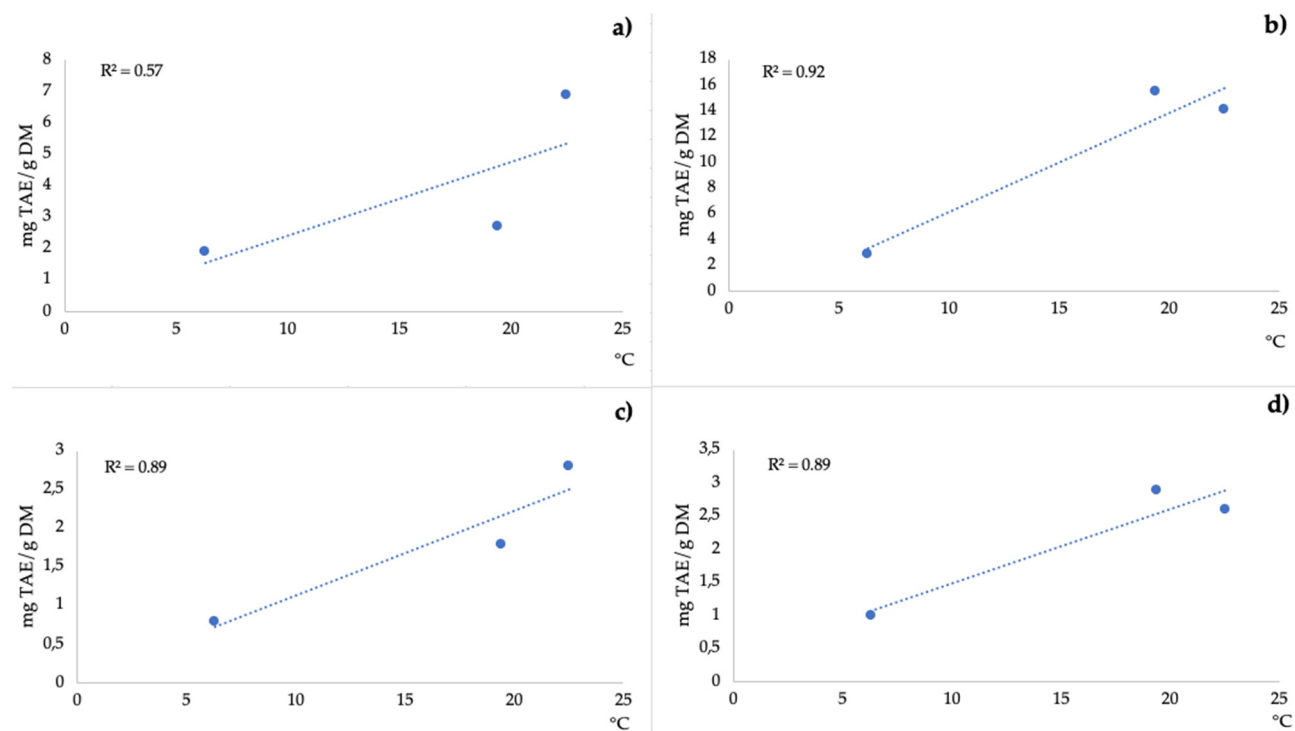




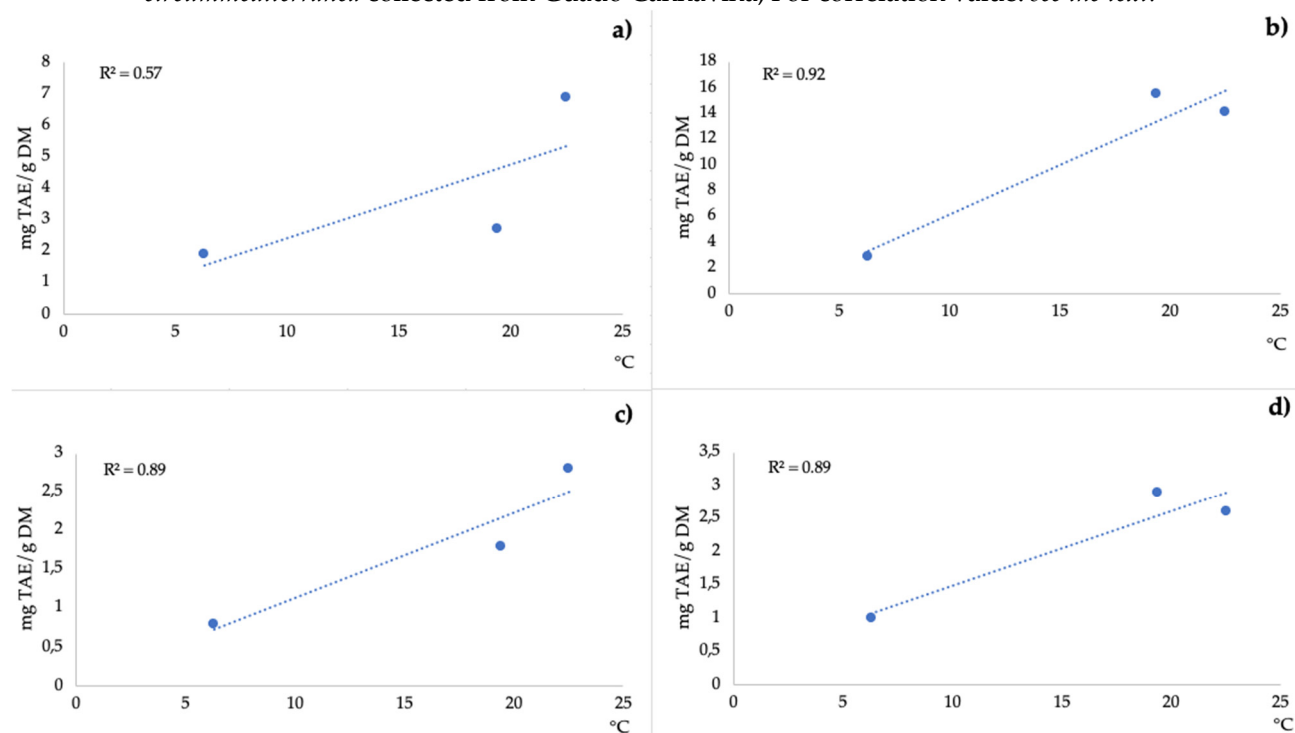
**Figure S2.** Correlation between the total polyphenols content and the sampling period, considering the mean temperatures of each sampling time. Please note: the correlation has been evaluated only when plant species was present during all period of sampling. **a)** *B. erectus* collected from Monteforte; **b)** *T. longicaulis* collected from Verrino; **c)** *F. circummediterranea* collected from Guardata; **d)** *F. circummediterranea* collected from Guado Cannavina; For correlation value: see the text.



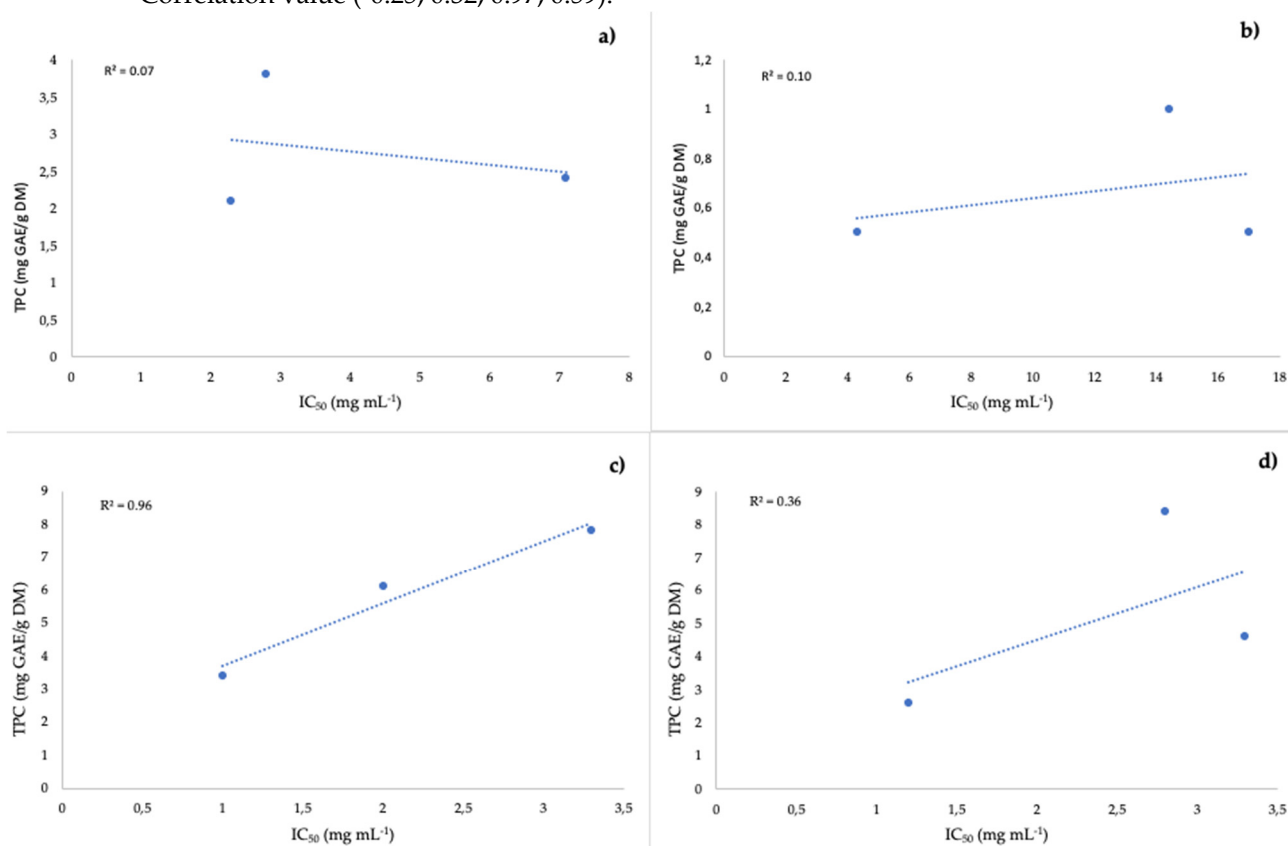
**Figure S3.** Correlation between the total tannins content and the sampling period, considering the mean temperatures of each sampling time. Please note: the correlation has been evaluated only when plant species was present during all period of sampling. **a)** *B. erectus* collected from Monteforte; **b)** *T. longicaulis* collected from Verrino; **c)** *F. circummediterranea* collected from Guardata; **d)** *F. circummediterranea* collected from Guado Cannavina; For correlation value: see the text.



**Figure S4.** Correlation between the condensed tannins content and the sampling period, considering the mean temperatures of each sampling time. Please note: the correlation has been evaluated only when plant species was present during all period of sampling. **a)** *B. erectus* collected from Monteforte; **b)** *T. longicaulis* collected from Verrino; **c)** *F. circummediterranea* collected from Guardata; **d)** *F. circummediterranea* collected from Guado Cannavina; For correlation value: see the text.



**Figure S5.** Correlation between the antioxidant activity and total polyphenols content of **a)** *B. erectus* collected from Monteforte; **b)** *T. longicaulis* collected from Verrino; **c)** *F. circummediterranea* collected from Guardata; **d)** *F. circummediterranea* collected from Guado Cannavina during all sampling periods. Correlation value (-0.25; 0.32; 0.97; 0.59).



The correlation between antioxidant activity and total polyphenol content is absent in the case of *B. erectus*, *T. longicaulis* and *F. circummediterranea*. A positive correlation was found between the total polyphenol content and the IC<sub>50</sub> value was found for *F. circummediterranea* (collected from Guardata), indicating that an increase in the total polyphenol content corresponds to a reduction in the antioxidant activity.