regression testing, there was a statistically significant relationship between the methods in aqueous phase ($r > 0.92$ and $r^2 > 0.84$, $p < 0.0001$, $n = 17$) (Figure 3). However, the TBARS method results did not correlate with the results of the other methods. There was no statistically significant relationship between TBARS and aqueous phase methods at the 95% confidence level ($|r| < 0.15$, $n = 10$). These

Table 1. Antioxidant activity of catechins, procyanidins, anthocyanins and pyranoanthocyanins. Each value represents the mean and standard deviation of three determinations.

	% inhibition of tyrosine nitration	TEAC	FRAP	IC$_{50}$ value (μM)
Catechins and procyanidins				
Catechin	45.1 ± 1.6	2.85 ± 0.12	1.08 ± 0.03	3.1 ± 0.2
Epicatechin	44.1 ± 3.7	2.93 ± 0.02	1.10 ± 0.02	3.2 ± 0.2
Gallocatechin	44.3 ± 2.9	3.31 ± 0.09	1.74 ± 0.04	15.9 ± 3.0
Epicatechin-3-*O*-gallate	66.0 ± 1.2	5.31 ± 0.38	3.10 ± 0.12	15.7 ± 2.1
Dimer B1 (Ec 4-8 cat)	72.3 ± 3.8	6.29 ± 0.09	3.15 ± 0.03	4.6 ± 1.0
Dimer B2 (Ec 4-8 Ec)	79.1 ± 2.3	8.36 ± 0.48	3.05 ± 0.03	3.6 ± 1.1
Dimer B3 (cat 4-8 cat)	59.1 ± 5.0	5.59 ± 0.10	2.39 ± 0.02	4.8 ± 1.5
Dimer B4 (cat 4-8 Ec)	63.9 ± 2.5	6.03 ± 0.25	2.75 ± 0.01	5.3 ± 1.9
Dimer B7 (Ec 4-6 cat)	59.2 ± 1.2	4.37 ± 0.04	1.84 ± 0.03	5.1 ± 0.6
Trimer (Ec-Ec-cat)	90.6 ± 0.9	8.60 ± 0.56	4.35 ± 0.03	6.7 ± 3.1
Anthocyanins				
delphinidin-3-monoglucoside	45.0 ± 6.8	2.93 ± 0.05	2.05 ± 0.02	N.D.
malvidin-3-monoglucoside	45.3 ± 4.8	2.45 ± 0.03	1.54 ± 0.02	N.D.
petunidin-3-monoglucoside	26.2 ± 1.4	1.96 ± 0.08	1.12 ± 0.03	N.D.
cyanidin-3-rutinoside	49.5 ± 5.4	2.77 ± 0.08	1.76 ± 0.08	N.D.
malvidin-3,5-diglucoside	39.6 ± 3.7	1.92 ± 0.05	1.61 ± 0.01	N.D.
Pyranoanthocyanins				
dephinidin-3-monoglucoside pyruvic acid adduct	33.7 ± 1.3	2.52 ± 0.05	1.31 ± 0.08	N.D.
malvidin-3-monoglucoside pyruvic acid adduct	38.4 ± 1.0	1.02 ± 0.07	0.72 ± 0.05	N.D.

N.D. – Not determined.

results could be explained, apart from the different hydrophilic or lipophilic properties of the substratum used in the different methods, by the different mechanism of antioxidant action measured for them.

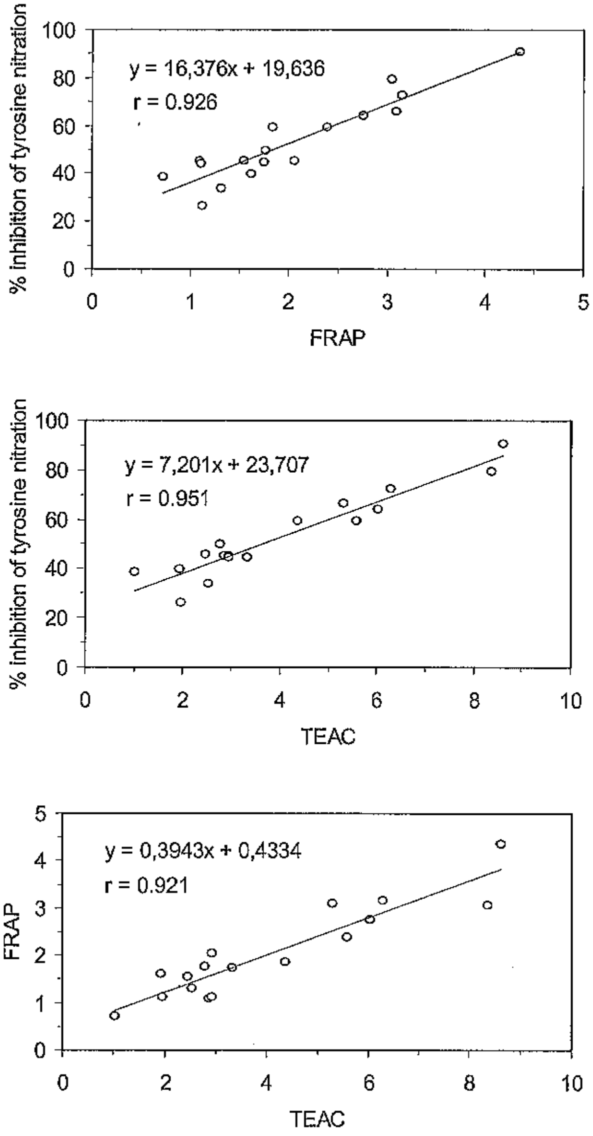

Figure 3. Relation between antioxidant activity measurements of 17 wine polyphenols using different methods (peroxynitrite scavenging activity, TEAC and FRAP).

It is evident that the expression of the results of antioxidant power of catechins, procyanidins, anthocyanins and pyranoanthocyanins, as well as the effect of diglycosylation of anthocyanins, depends on the assay used and, therefore, also the mechanisms of these methods used to evaluate the antioxidant activity. The TEAC, FRAP and inhibition tyrosine nitration methods can be recommended to evaluate the antioxidant capacities of wine polyphenols (procyanidins, anthocyanins and pyranoanthocyanins). Nonetheless, the TBARS assay is not recommended for anthocyanins and their adducts. In general, it could be concluded that procyanidins were, among the tested groups, the ones which showed more antioxidant capacity in the distinct aqueous methods used, followed by anthocyanins and pyranoanthocyanins.

3. Experimental Section

3.1 Chemicals and reagents

(+)-catechin (cat), (-)-epicatechin (Ec), (-)-epicatechin-3-*O*-gallate, gallocatechin and malvidin-3,5-diglucoside were obtained from Sigma-Aldrich (Steinhein, Germany). Butylated hydroxytoluene (BHT) was purchased from Aldrich (Milwaukee, USA), 2,2′-azinobis-(3-ethyl-benzo-thiazoline-6-sulphonate) (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox C), 2,4,6-tri(2-pyridyl)-1,3,5-pyridyltriazine (TPTZ) and trichloracetic acid (TCA) were obtained from Fluka Chemie (Buchs, Switzerland), thiobarbituric acid (TBA), phosphatidylcholine and myoglobin were obtained from Sigma (St. Louis, USA) and $FeCl_3$ was obtained from Panreac Química SA (Barcelona, Spain). All other chemicals used were of analytical grade.

3.2 Isolation and purification of procyanidins

Procyanidin dimers B2, B3 and B4 were isolated from grape seeds; procyanidins of the B1 series (B1, B7 and trimer Ec-Ec-cat) were from almond fruit flesh. The procyanidins were isolated as previously described by Plumb et al., [25]. Purities of dimers and trimers used in this work, which were tested by HPLC in the photodiode apparatus, selecting 280 nm as the preferred wavelength, were > 90%.

3.3 Isolation and purification of anthocyanins

Anthocyanins (delphinidin-3-monoglucoside, malvidin-3-monoglucoside, petunidin-3-monoglucoside and cyanidin-3-rutinoside) were isolated from a methanol-acid red grape skin extract by semipreparative HPLC using a Waters 600 chromatograph. The column was Ultracarb ODS (5 μm, 250 × 10 mm) (Phenomenex). The solvents were 5% acetic acid (A) and methanol (B) applied with the following gradient: from 10% to 15% B for 15 min, isocratic 15% B for 5 min, from 15% to 30% B for 30 min, from 30% to 45% B for 10 min, from 45% to 10% B for 10 min at a flow rate of 3 ml/min. Detection was carried out at 520 nm.

3.4 Pyranoanthocyanins syntheses

Syntheses of dephinidin-3-monoglucoside pyruvic acid adduct and malvidin-3-monoglucoside pyruvic acid adduct were performed as previously described by Romero et al., [26]. Pyruvic acid was added to pure anthocyanin, dissolved in potassium hydrogen tartrate buffer containing 10% ethanol in a molar ratio of pyruvic acid to pure anthocyanins of 300:1. The pH was adjusted to 3.7 by addition of Na_2CO_3 and the solution was incubated at 32 °C in the dark in the presence of air.

The purity of anthocyanins and pyranoanthocyanins was tested by HPLC using the method of de Pascual-Teresa et al., [27] with minor modifications. Briefly, a Hewlett-Packard 1100 HP system equipped with a quaternary pump and photodiode array detector was used. The column was an Aqua® C18, 5 μm (150 × 4.6 mm) (Phenomenex), thermostatted at 35 °C. The solvents were 0.1%