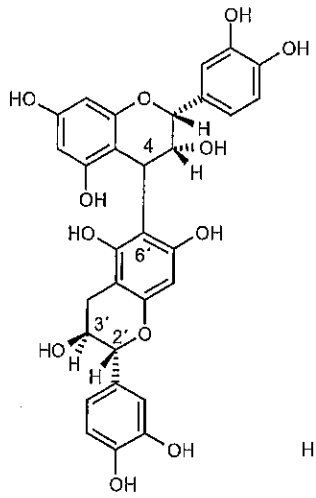
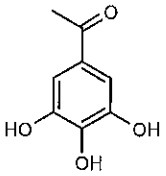
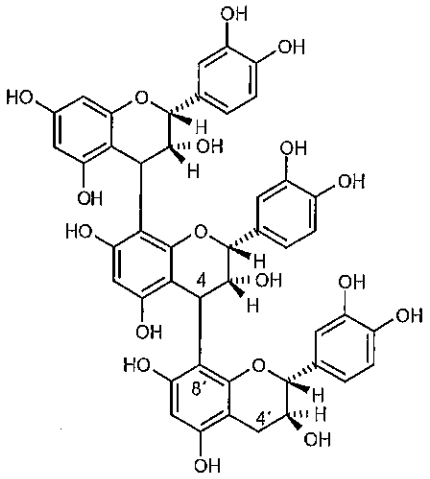
which increased from monomer to trimer. Galloylation reduced the ability to prevent peroxidation of phosphatidylcholine vesicles.



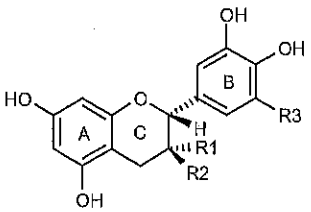
B7



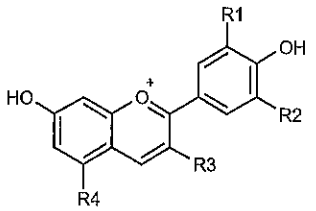
G: Galloyl



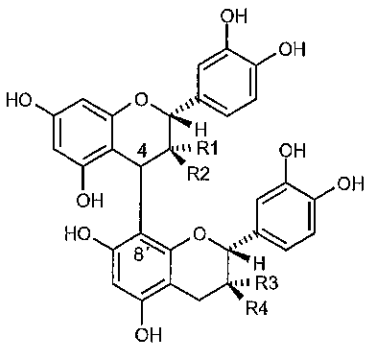
EEC



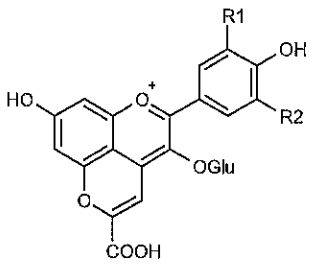
(+)-Catechin: R1=H; R2=OH; R3=H
(-)-Epicatechin: R1=OH; R2=H; R3=H
(+)-Gallocatechin: R1=H; R2=OH; R3=OH
(-)-Epicatechin-3-O-gallate: R1=OG; R2=H; R3=H



Delphinidin-3-Glu: R1=OH; R2=OH; R3=OGlu; R4=OH
Cyanidin-3-Rut: R1=OH; R2=H; R3=ORut; R4=OH
Petunidin-3-Glu: R1=$OCH_3$; R2=OH; R3=OGlu; R4=OH
Malvidin-3-Glu: R1=$OCH_3$; R2=$OCH_3$; R3=OGlu; R4=OH
Malvidin-3,5-diGlu: R1=$OCH_3$; R2=$OCH_3$; R3=OGlu; R4=OGlu



B1: R1=OH; R2=H; R3=H; R4=OH
B2: R1=OH; R2=H; R3=OH; R4=H
B3: R1=H; R2=OH; R3=H; R4=OH
B4: R1=H; R2=OH; R3=OH; R4=H



Delphinidin-3-Glu pyruvic acid adduct: R1=OH; R2=OH
Malvidin-3-Glu pyruvic acid adduct: R1=$OCH_3$; R2=$OCH_3$

**Figure 1.** Structures of catechins, procyanidinds, anthocyanins and pyranoanthocyanins. Glu, glucoside; Rut, rutinoside.

Nonetheless, in the aqueous phase methods, galloylation resulted in a significant increase of antioxidant activity. Hydroxylation of catechin to gallocatechin did not show a considerable effect in the inhibition of tyrosine nitration, however, it presented a significant increase in antioxidant effectiveness in the TEAC and FRAP assays, in contrast to the lipid phase where the antiradical activity was significantly reduced. The antioxidant activity of the tested series of polyphenolic compounds correlated with the number of aromatic hydroxyl groups in the aqueous phase assays. Different results were found in the lipid phase system where the trend of a decrease in the antioxidant efficiency with the number of aromatic hydroxyl groups was observed. These differences between aqueous and lipid phase methods are in agreement with previously published data [19], where the results between the aqueous (TEAC method) and lipid phase system (TBARS method) for catechins and procyanidins were compared. Nevertheless, the present work provides more information about these features, since two more antioxidant methods (FRAP and peroxynitrite scavenging activity) were used to analyze the antioxidant capacity of these compounds. According to Auroma [20], the use of more than one method is recommended in the study of antioxidant capacity, because it is clear that no single method can give a comprehensive prediction of antioxidant efficacy.

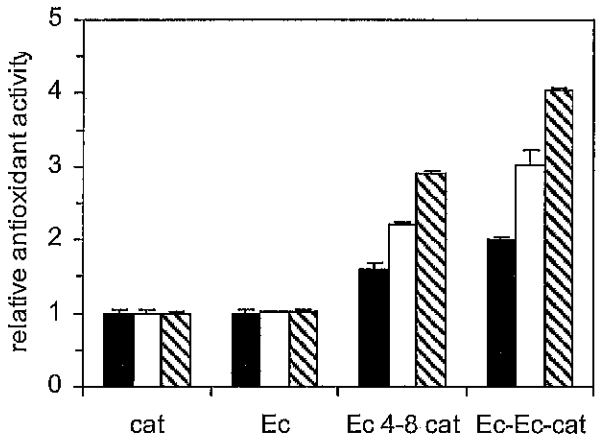



**Figure 2.** Influence of polymerisation of catechins on antioxidant activities in aqueous phase systems; peroxynitrite scavenging activity (■), TEAC (□), FRAP (▨). Values are expressed relative to catechin activity. Each value represents the mean and standard deviation of three determinations.

The activities of anthocyanin monoglycosides tested in TEAC and FRAP assays decreased in the order: delphinidin-3-monoglucoside > cyanidin-3-rutinoside > malvidin-3-monoglucoside > petunidin-3-monoglucoside. For the structure activity relationship study we did not have cyanidin-3-monoglucoside and for this reason we decided to test cyanidin-3-rutinoside. The completely conjugated structure of anthocyanins, which allows electron delocalization, results in very stable radical products, which is favorable for antioxidative capacity. The degree and position of hydroxylation and methoxylation in the B ring affect stability and reactivity and, thereby, also antioxidant actions. The TEAC and FRAP assays results for antioxidant activity of anthocyanins are in accordance with the previous observations of the effect of hydroxylation and methoxylation in ring B studied by DPPH radical scavenging capacity [9]. The anthocyanins malvidin-3-glucoside and petunidin-3-glucoside showed lower efficiency compared to cyanidin-3-rutinoside and delphinidin-3-glucoside. The third

hydroxyl group in the B ring enhanced the activity, as delphinidin-3-monoglucoside with hydroxyl groups in the 3′-, 4′-, and 5′-positions was significantly more effective than cyanidin-3-rutinoside with hydroxyl groups in 3′- and 4′-positions. Moreover, in this study, different glycosylation patterns may have modified the antioxidant and antiradical activities of the anthocyanins. Kähkönen et al., showed that delphinidin and cyanidin-3-rutinoside are less active in the DPPH scavenging activity than the corresponding monoglucosides, although this effect is very much dependent on the method used [9]. For the FRAP and TEAC assays the methoxylation of hydroxyl groups in 5′ (petunidin-3-monoglucoside) or 3′ and 5′ positions (malvidin-3-monoglucoside) significantly reduced the antioxidant activity. However, the antioxidant activity showed by malvidin-3-monoglucoside in peroxynitrite mediated tyrosine nitration was the same as that of delphinidin-3-monoglucoside and cyanidin-3-rutinoside activities. The activity of anthocyanins in preventing tyrosine nitration decreased in the following order: cyanidin-3-rutinoside > malvidin-3-monoglucoside ≈ delphinidin-3-monoglucoside > petunidin-3-monoglucoside. Significant differences were not found between delphinidin-3-monoglucoside, cyanidin-3-rutinoside and malvidin-3-monoglucoside, but significant differences were found between the above mentioned compounds and petunidin-3-monoglucoside. Once more, these results show the importance of the substitution groups in the B ring of the anthocyanin molecules. Tsuda et al. [21] demonstrated the mechanisms which pelargonidin, anthocyanidin with one hydroxyl group on the B ring, scavenge the $ONOO^-$. First, pelargonidin is broken by the radical and *p*-hydroxybenzoic acid is then formed. Later, this acid reacts with $ONOO^-$, which results in the formation of 4-hydroxy-3-nitrobenzoic acid. It is probable that the different results among the glucosides of anthocyanidins are due, on the one hand, to different mechanisms from pelargonidin to protect against the peroxynitrite-mediated nitration of tyrosine [21] and on the other hand that the different acids formed by the reaction between the anthocyanins and the radical present different affinities to $ONOO^-$.

Anthocyanins may exist in a variety of protonated, deprotonated, hydrated, and isomeric forms and the relative proportion of these molecules is strongly dependent on pH. These forms may play an important role in the antioxidant activity. Moreover, the peroxynitrite anion ($ONOO^-$) and its conjugate acid (HOONO) could have different reactivities [22] and the relative proportion is too strongly dependent on pH ($pK_a$ = 6.8). Inhibitions of tyrosine nitration were measured at pH 6.0 (≈ 15% of peroxynitrite was in the anionic form) (Table 1) and a comparative study at physiological pH (7.4) was performed to be more representative of biological conditions. The peroxynitrite scavenging activity of anthocyanins at pH 7.4 (≈ 80% of peroxynitrite was in the anionic form) decreased in the following order: cyanidin-3-rutinoside > malvidin-3-monoglucoside ≈ delphinidin-3-monoglucoside > petunidin-3-monoglucoside (data not shown).

The glycosylation of malvidin-3-monoglucoside to malvidin-3,5-diglucoside caused significant reduction of the antioxidant power in the TEAC assay, but had no significant effect on the inhibition of tyrosine nitration, though the FRAP value for malvidin-3,5-diglucoside was higher than for malvidin-3-monoglucoside (Table 1).

As it can see in table 1, the incorporation of pyruvic acid into delphinidin-3-monoglucoside and malvidin-3-monoglucoside caused a significant decrease in antioxidant activity in aqueous phase assays. These results are in agreement with previously published data [23,24].

The correlation coefficients between the results obtained for inhibition of tyrosine nitration, FRAP, TEAC and TBARS assays were examined for all the tested compounds. On the basis of simple