Journal of Biophysical Chemistry, 2019, 10, 1-14
http://www.scirp.org/journal/jbpc
ISSN Online: 2153-0378
ISSN Print: 2153-036X

In Silico and *in Vitro* Approach for the Understanding of the Xanthine Oxidase Inhibitory Activity of Uruguayan Tannat Grape Pomace and Propolis Poliphenols

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How to cite this paper: Alvareda, E., Iribarne, F., Espinosa, V., Miranda, P., Santi, D., Aguilera, S., Bustos, S. and Zunini, M.P. (2019) *In Silico* and *in Vitro* Approach for the Understanding of the Xanthine Oxidase Inhibitory Activity of Uruguayan Tannat Grape Pomace and Propolis Poliphenols. *Journal of Biophysical Chemistry*, **10**, 1-14.
https://doi.org/10.4236/jbpc.2019.101001

Received: November 27, 2018
Accepted: February 25, 2019
Published: February 28, 2019

Abstract

The use of food additives with xanthine oxidase (XO) inhibitory activity offers an alternative approach to hyperuricemic and gout disease treatment, and provides an example of antioxidant nutraceutics. The *in vitro* and *in silico* XO inhibitory activity of polyphenols from Uruguayan Tannat grape pomaces and propolis extracts was evaluated as well as the scavenging capacity of said compounds. When comparing propolis and grape pomace samples, the *in vitro* studies demonstrated that polyphenols extracted from propolis are more active as free radical scavengers than those from Tannat grape pomace. Both natural products effectively inhibited XO but the capacity of phenols present in GP is higher than the one present in P. The high content of anthocyanins in GP, absent in P, could account for this observation. *In silico* assays allowed us to determine relevant ligand-receptor interactions between polyphenols, from a database built with previously reported polyphenols from both natural products, and the active site of XO. The *in silico* results showed that compound (E)-isoprenylcaffeate from propolis was the best potential XO inhibitor displaying hydrophobic aromatic interaction between the conjugated ring of the caffeate moiety and polar interactions between hydroxyl groups from caffeate with the active site polar residues. Among grape po-

maces, the Cyanidin-3-O-(6-(E)-p-coumaroyl)-glucoside was the best XO inhibitor; its moiety oxychromenyl being relevant to the docking stabilization. All these results lead us to propose Uruguayan propolis and Tannat grape pomace extracts as food additives as well as phytopharmaceuticals to decrease the uric acid levels in gout disease and to act against oxidative stress.

Keywords

Propolis, Tannat Grape Pomace, Xanthine Oxidase Inhibition, Polyphenols, Functional Foods

1. Introduction

Xanthine oxidase (XO) participates in purine catabolism, generating uric acid as a final product and the release of reactive oxygen species (ROS) such as $O_2^{\bullet -}$ and H_2O_2. The exacerbated synthesis of uric acid causes the development of gout disease [1] [2]. At the same time, ROS are responsible for triggering a lot of disorders such as atherosclerosis, ageing, cancer, ulcer and inflammation [3] [4] [5] [6] [7]. Current gout treatments are palliative at the level of pain and chronic inflammation, and although treatments involving xanthine oxidase inhibitors are available, serious adverse effects have been reported [8]. In this context, natural products represent an important alternative. In particular, numerous polyphenols have been reported as relevant XO inhibitors [1] [2] [9] [10] [11] [12]. In addition, recent *in vitro* and *in silico* reports on XO inhibition by quercetin (a natural flavonoid) gave consistent and promising information to consider this natural flavonoid as a relevant compound for preventing gout and oxidative damage [13]. The antioxidant action mechanism of quercetin and its role on the inhibition of XO catalysis was also proposed. In this sense, the binding site for quercetin was mapped near the molybdopterin cofactor where the oxidation of xanthine (natural substrate) takes place. Thereafter, the reduction of the substrate oxygen is mediated by the isoalloxazine ring of the flavin adenine dinucleotide (FAD) center, transferring electrons and generating ROS like superoxide radical anion ($O_2^{\bullet -}$) or hydrogen peroxide (H_2O_2) [13] [14].

Propolis (P) is a natural resinous product elaborated by honeybees (*Apis mellifera*) that displays a lot of biological activities such as antibacterial, antiviral, antifungal, anti-inflammatory, anticancer, and antioxidant [15] [16].

Grape pomaces (GP) are a by-product of wine industry and consist mainly of peels (skins), seeds and stems. Antioxidant, antimicrobial, antimutagenic, anticarcinogenic and antilipogenic activities have been reported for GP [17] [18].

Both P and GP, have been described as important sources of bioactive compounds, most of them polyphenols [17] [19] [20] [21] [22]. In this context, Yoshizumi *et al.* [16] suggested that a continuous intake of Chinese propolis may be effective for the prevention and the treatment of gout and hyperuricemia.

Furthermore, ethanolic extracts from grape pomace increase the antioxidant properties of wines [18].

In this work, we studied the total phenolic contents, the scavenging activity and, for the first time, the *in vitro* XO inhibitory activity of polyphenolic rich extracts from Uruguayan propolis and Tannat grape pomace. In addition, *in silico* assays were performed in an attempt to account for the molecular basis of the interaction between the enzyme and polyphenols-like compounds, present in the extracts according to previous reports [23] [24].

2. Materials and Methods

2.1. General

Folin-Ciocalteu' phenol reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), gallic acid, ascorbic acid and sodium carbonate (Na_2CO_3) were obtained from Sigma (Sigma-Aldrich® GmbH, Sternheim, Germany). Methanol was obtained from Merck®. All other chemicals used were of analytical grade. For the XO inhibition, a Sigma-Aldrich Xanthine Oxidase Activity Assay Kit was used (cat. MAK078, Sigma-Aldrich®, St. Louis, MO). Measures were obtained from Elisa Thermo® Scientific Labsystems Multiskan™ RC.

2.2. Samples and Extracts

P and GP sampling and the respective extraction procedures were developed by our group and previously published [23] [24]. Briefly, propolis and Tannat grape pomace samples were collected in different zones form the south region of Uruguay.

2.2.1. Propolis Extracts

Samples of 1g weight were separately extracted. Waxes were removed by Soxhlet in 200 mL hexane during one hour and 12 refluxes. After evaporating the hexane and drying, they were grinded and phenols were extracted with ethanol-water (80 - 20 v/v) during one hour at a 75˚C. Finally, 25 mL of extract were filtered and diluted to 50 mL with the same extraction solvent. The liquid extracts were stocked in dark at 4˚C until their analysis.

2.2.2. Grape Pomace Extracts

Frozen grape pomace samples were submitted during 24 hours to drying in vacuum at a temperature of 60˚C. The dry grape pomace samples were grinded and stocked in darkness. Phenols were extracted by reflux in a mixture of ethanol-water (80 - 20 v/v) during 2 hours at 50˚C. After filtering, the extracts were conserved at 4˚C in darkness until their use.

2.3. Total Phenolic Content (Folin-Ciocalteau)

Total phenolic content was determined by means of Folin-Ciocalteau reagent using the method described by Singleton *et al.* [25]. The results are reported as mean values of GAE (gallic acid equivalents) in μM, using the calibration curve

prepared with gallic acid standard solutions.

2.4. DPPH Radical Scavenging Activity

Free radical scavenging activities of extracts were analyzed, based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The absorbance was measured at 517 nm; the procedure was the same described in Shimada *et al.* [26]. Acid ascorbic was used as a positive control and the results are reported as mean values of grams of ascorbic acid equivalents per 100 g dry extract.

2.5. *In Vitro* XO Inhibition Assays

The Xanthine Oxidase activity assay kit was used according to the manufacturer instructions. The measure of XO activity was performed by an enzyme assay, leading to a colorimetric (570 nm) blue product, proportional to the generated hydrogen peroxide (cat. MAK078, Sigma-Aldrich®, St. Louis, MO). Assays were performed in 15 min according to supplier instruction using 5 µL of samples in triplicate.

2.6. Xanthine Oxidase *in Silico* Assays

2.6.1. *In Silico* General Tools and Modeling

All *in silico* calculations and procedures were performed with the Molecular Operating Environment MOE™, 2015.10 [27]. To build the database, 123 phenolic structures from bibliographic sources [20] [24] [28] [29] [30] [31] were modeled and refined by energy minimization, using the MMF94x force field [32]. The resulting phenolic database was previously reported [24].

The X-ray structure of mammalian xanthine oxidase complexed with quercetin (flavonoid inhibitor) at 2.0 Å resolution was obtained from the RCSB Protein Data Bank (PDBid: 3NVY) [14].

2.6.2. Xanthine Oxidase Docking Procedure

The aforementioned crystallographic structure of the XO-quercetin complex was used as starting point for the docking procedure. As expected, one of the detected sites was the active site which accommodates quercetin, the same site identified by Hille *et al.* [33] next to the molybdopterin cofactor. The benzopyran moiety of quercetin is oriented between Phe914 and Phe1009 residues and the B ring is next to the molybdopterin cofactor named MTE1327-MOS1228, at the active site [14] [33].

For the docking calculations, a 4.5 Å sphere centered on quercetin was considered. The docked quercetin pose overlapped nicely the ligand crystallographic pose, with a 2.8 Å root mean square deviation (RMSD) when Alpha Triangle (placement), Affinity ΔG (scoring) and final force field (MMFF94x) refinement were used. The docking scores were calculated with the Affinity ΔG function. This scheme had been developed previously by us [12] [23] [24]. The highest ranked poses were evaluated using the score and the relative position with re-

spect to the native ligand [14]. The graphical representations of the calculated binding poses were performed by Surface Maps, and Ligand Interaction MOE™ tools [34].

3. Results and Discussion

The results for the different samples of Uruguayan propolis and tannat gape pomaces are summarized in **Table 1**. All samples were collected from the south of Uruguay near Montevideo and then analyzed, to determine the amount of phenolic compounds. Phenolic contents were obtained by Folin-Ciocalteu and they are expressed as GAE (gallic acid equivalents) in μM. DPPH. Radical scavenging activities are expressed as AEAC (ascorbic acid equivalent antioxidant capacity) in μM. **Table 1** also presents the *in vitro* XO inhibitory activity (%) of all samples.

3.1. Total Phenolic Content (Folin-Ciocalteau)

Both types (e.g. propolis and grape pomaces) of hydroalcoholic extracts showed different amount of polyphenols with the same extraction mixture. Phenolic contents of propolis extracts are higher (mean = 10.9 ± 0.3 GAE μM), than those of grape pomace (mean = 5.5 ± 0.9 GAE μM), evidencing that propolis extracts present more polyphenolic content (**Table 1**). The GP 2010 Tannat sample (extracted in 2013) presented fewer phenols than the Tannat sample extracted in the same year that was collected (GP 2013), suggesting that the amount of polyphenols from grape pomace samples probably decreases with aging.

3.2. DPPH Radical Scavenging Activity and Xantine Oxidase *in Vitro* Assays

The evaluation of the antioxidant potential from the extracts was performed by the DPPH radical scavenging method. The results depicted in **Table 1** show that the extracted polyphenols from propolis are far more active as free radical scavengers than those from grape pomace extracts. However, XO inhibition of P extracts is only slightly higher than that of GP ones (mean values of 44% and 36%, respectively). Considering that the total amount of phenols in GP is half the one measured in P, it may be suggested that the specific potency of phenols present in GP is higher than the capacity of those phenols present in P. In particular, the high content of anthocyanins in GP could account for this observation.

This hypothesis is further substantiated by previous studies that found that anthocyanins were effective XO inhibitors [18] [35] [36] [37]. At the same time, other works reported that anthocyanins are present in GP extracts [17] [19] [23] [24]. But absent in P extracts [23] [24] [38].

Previously, it was also reported that, while the concentration of polyphenols remains constant after wine maturation, the anthocyanins concentration is diminished [39]. These finding may be also correlated with our observations in GP

Table 1. Phenolic content (μM GAE), radical scavenger activity (μM DPPH) and XO *in vitro* inhibitory activity (%) of different samples (obtained in the years indicated) of Uruguayan propolis (P) and Tannat grape pomaces (GP).

Sample code	GAEa μM + SD	DPPHb μM + SD	XO inhibition activity (%) + SD
P1 2008	10.4 + 0.2	---	68.8 + 0.9
P2 2008	12.3 + 0.1	37.0 + 0.0	41.7 + 0.0
P3 2008	14.3 + 0.1	37.3 + 0.2	68.1 + 0.6
P4 2009	11.0 + 0.1	36.9 + 0.5	39.6 + 0.9
P5 2009	13.2 +0.1	37.8 + 0.1	20.8 + 0.4
GP 2010 Tannat (extracted in 2013)	3.3 + 0.5	1.9 + 1.4	15.3 + 0.5
GP 2013 Tannat	7.7 + 1.2	10.3 + 1.4	56.3 + 0.9
P6 2013	4.0 + 0.2	16.2 + 1.0	25.0 + 0.4

[a]Gallic acid equivalents, [b]Ascorbic acid equivalent antioxidant capacity (AEAC). Propolis samples were labeled with a consecutive number and with their collected and extracted year. Year of collection and extraction was the same for all samples except for sample GP 2010 which was collected in 2010 but extracted in 2013. All samples were collected from the South of Uruguay.

extracts: the decrease in XO inhibitory activity through time could be due to the decrease in anthocyanins concentration. However, more thorough studies are necessary to prove this hypothesis correct.

The *in vitro* results suggest that the extracted compounds are responsible for the XO inhibitory activity and, in turn, would be able to cause the decline in the amount of free radicals and the uric acid deposits. Indeed, the events mediated by polyphenols might cause a decrease in the evolution of gout disease, as well as the tissue damage reduction usually observed in other pathologies associated with an increase in ROS generation such as ischemia-reperfusion among others [40].

3.3. Xanthine Oxidase *in Silico* Data Analysis

Table S1 (**Supplementary Material**) lists the 60 best docked scores from the 123 phenolic compounds database previously found in propolis [15] [28] [29] and grapes [20]. This very same database was previously reported by us [24]. 52 of the 60 compounds belong to GP extracts. This is consistent with the *in vitro* results observed for GP and P extracts from the same year, which show that the former presented the best inhibition.

3.4. Docking Calculations

The best docked compound, the hydroxycinnamic acid derivative, ester (E)-isoprenylcaffeate (score = −5.7884 kcal·mol^{-1}) belonged to propolis extracts. When a ligand interaction plot was performed for this compound, an interesting π-π stacking interaction was observed between the caffeate moiety and Phe914 at a distance of 3.75 Å and next to cofactor molybdopterin (molybdenum center),

see Figure 1. This interaction stabilizes the structure; a similar stacking was described by Wróblewski *et al.* [41] and by Cao *et al.* [14]. The isoprenyl portion appears exposed to the solvent (Figure 1).

In the Tannat GP extracts, the best docked compound was the anthocyanin named cyanidin-3-O-(6-(E)-p-coumaroyl)-glucoside. In Figure 2, the most important interactions with the XO active site are depicted. The same π-π stacking interaction found for the binding of ester (E)-isoprenylcaffeate was also verified here but now between the coumaroyl moiety and Phe914 at a distance of 3.70 Å (also next to cofactor molybdopterin (molybdenum center). A hydrogen bonding with Thr1010 and an ionic interaction with Glu879 were also observed.

The results for both compounds in Figure 1 and Figure 2 suggest that the acceptor hydrogen bond interactions with the active site residue side chains, plus the hydrophobic factor embodied by π-π interactions between Phe914 and phenolic rings, play an important role in XO inhibition mechanism, as was previously suggested by Cao *et al.* [14]. Also, a hydrophilic environment seems to be preferred for the inhibitors, next to the molybdenum center near Arg880, Ser876, Glu802 or Glu1261.

The comparison between the *in silico* results and the *in vitro* experiments for XO inhibition would endorse our previous hypothesis in relation to the importance of anthocyanins. In effect, even allowing for the fact that the score differences are rather small across all compounds in Table S1, anthocyanins represent over 60% of the 60 best docked compounds in XO, and half of the 10 best compounds.

4. Conclusions

XO inhibition and scavenging of free radicals are potentially a powerful strategy for hyperuricemia and the fight against oxidative stress.

In this work, we were able to prove that the Uruguayan P and Tannat GP extracts can act as free radical scavengers, with samples of propolis being significantly more active than those of grape pomaces. In addition, we have shown that P and GP samples are capable, to different extents, of inhibiting XO. However, XO inhibition by P extracts is only slightly higher than GP. Considering that the total amount of phenols in GP is half of that measured in P, we attribute these results to the anthocyanin-like compounds, only present in GP extracts. In other words, we believe that the superior inhibitor ability of GP extracts is mainly due to the high concentration of anthocyanins present in the early stages of the grape pomaces. Further experimental assays that measure the exact concentration of anthocyanins are necessary to confirm this hypothesis.

The molecular docking results were used to determine the way in which the phenolic compounds would interact with XO's active site. In effect, considering the results yielded by the best docked compounds, it can be concluded that, at the molecular level, three different types of interactions are established with the enzyme: a) hydrophobic aromatic interactions between the conjugated ring of

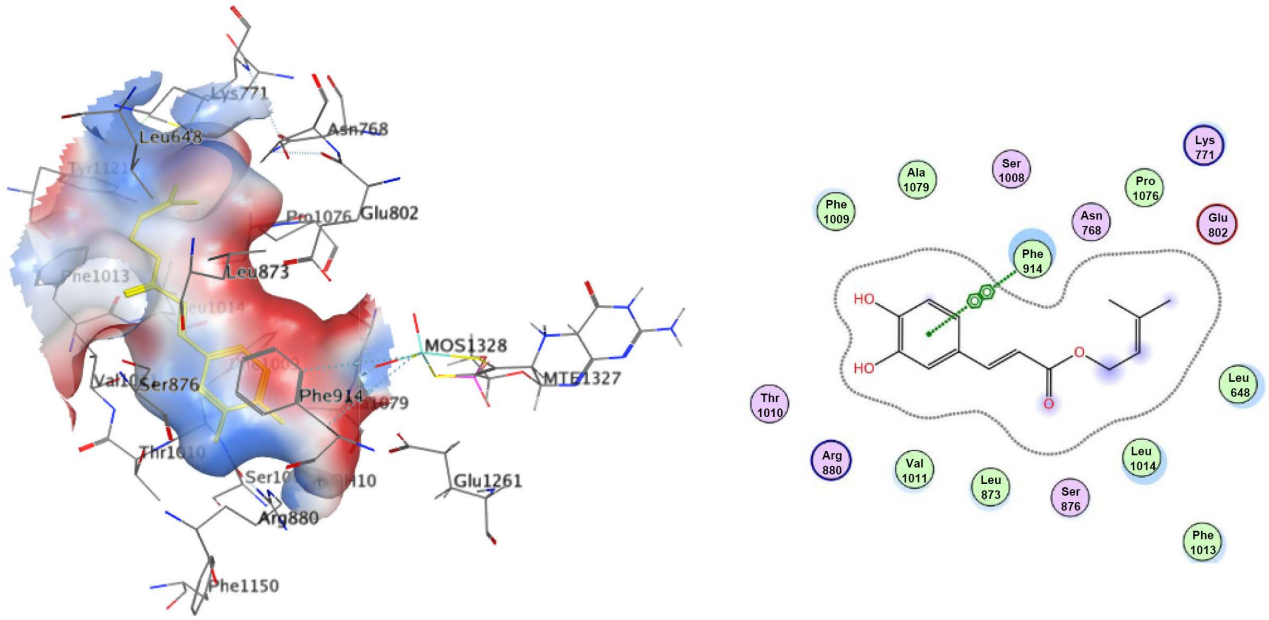

Figure 1. 2D Electrostatic Surface Map and Ligand Interaction of the best docked ligand from all samples, ester (E)-isoprenyl-caffeate (score = −5.7884 kcal·mol^{-1}) displayed in yellow.

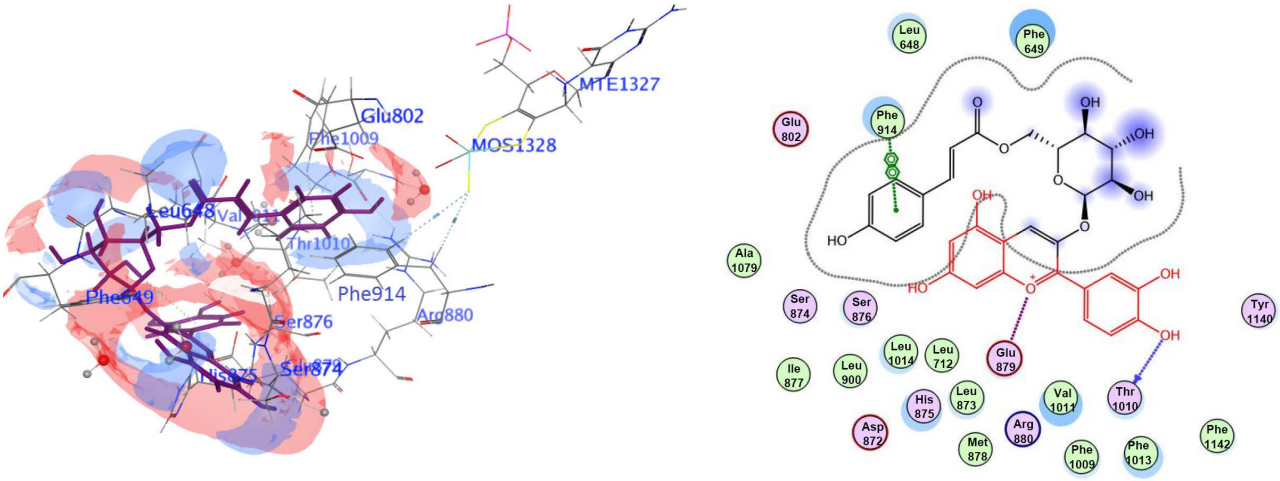

Figure 2. 2D Electrostatic Surface Map and Ligand Interaction of the best docked compound from GP extracts, Cyanidin-3-O-(6-(E)-p-coumaroyl)-glucoside, displayed in violet.

the caffeate moiety and the conjugated coumaroyl ring, with the active site phenylalanine residues; b) polar interactions between hydroxyl groups of caffeate and active site polar residues; c) the moiety oxychromenylium from anthocyanins which is relevant to the docking stabilization, and gives to this kind of compounds enough interaction capacity expressed in their higher scores. Also, important interactions with residues that are relevant to the catalytic function of the XO were observed.

The combination of *in vitro* and *in silico* techniques and procedures applied here to study the nature of enzyme inhibition mechanisms and biological activities helped us to comprehend the antioxidant functionality that a mixture of ex-

tracts obtained from two natural products may have. We believe that the synergy between both kinds of methodologies is fundamental for the discovering of new drugs, and in this case, of potential additives for functional foods. In effect, grape pomace could constitute a cheap source of dietary antioxidants because they are secondary products of wine manufacturing. In the case of propolis, this product is consumed in small quantities by the population, so these results could constitute a good opportunity to add value to it.

In summary, according to our results we postulate that, either by inhibiting XO or by the scavenging of free radicals, propolis and grape pomace extracts may reduce the amount of free radicals and uric acid deposits. Thus, these natural products may be considered as functional foods and good candidates to develop phytopharmaceuticals for gout disease treatment.

Acknowledgements

This work was supported by Proyecto de Desarrollo de Ciencias Básicas (PEDECIBA) and Comisión Sectorial de Investigación Científica (CSIC), Universidad de la República—Uruguay. The authors also wish to thank Profs. Karen Ovsejevi and Carmen Manta, Cátedra de Bioquímica-Facultad de Química, Universidad de la República—Uruguay and Bodegas Carrau S. A. forprovidingthe grape pomacesamples.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Information (SI)

Table S1. Xanthine oxidase docking scores (Affinity ΔG algorithm), of the 60 best docked compounds. A PubChem code as a compound identifier (CID) is given in parentheses, (*CID refers to a CID parent code). Origin of compounds denoted as "P" (Propolis) or "GP" (grapes). Basic structural scaffolds as follows: PHE AC: Phenolic acids; PROCY: Procyanidins; ANTHO: Anthocyanins; FLAV: Flavonoid; STYL: Stylbene; CAT: Catechin.

Name**	XO Scores (kcal·mol^{-1})	Origin**	Structure**
(E)-isoprenylcaffeate (5281790)	−5.7884	P	PHE AC Ester
Cyanidin-3-O-(6-(E)-p-coumaroyl)-glucoside (5282067)	−5.6533	GP	ANTHO
Pyruvic derivative of malvidin-3-O-glucoside (443652)	−5.3929	GP	ANTHO
Pyruvic derivative of delphinidin-3-O-(6-p-coumaroyl)-glucoside (15922818)	−5.3318	GP	ANTHO
(Z)-isoprenylcaffeate (5281790)	−5.3258	P	PHE AC Ester
Malvidin-3-O-(6-(E)-p-coumaroyl)-glucoside-4-vinylcatechol (*44256995)	−5.3088	GP	ANTHO
Petunidin-3-O-glucoside-4-vinylcatechol (*443651)	−5.1905	GP	ANTHO
(E)-phenethylcaffeate (CAPE) (5881787)	−5.1849	P	PHE AC Ester
5-methoxypinobanksin-3-O-pentanoate (147459)	−5.1234	GP	FLAV Ester
(E)-cinnamylcaffeate (5281787)	−5.0530	P	PHE AC Ester
Pinobanksin-3-O-acetate (148556)	−5.0341	P	FLAV Ester
Malvidin-3-O-(6-acetyl)-glucoside-4-vinylguaiacol (*44257037)	−4.9974	GP	ANTHO
Peonidin-3-O-glucoside-4-vinylguaiacol (*443654)	−4.9925	GP	ANTHO
Petunidin-3-O-(6-(Z)-p-coumaroyl)-glucoside (44256963)	−4.9893	GP	ANTHO
Malvidin-3-O-glucoside-4-vinylguaiacol (44257037)	−4.9663	GP	ANTHO
(E)-caftaric acid (6440397)	−4.9550	GP	PHE AC
Delphinidin-3-O-(6-(E)-p-coumaroyl)-glucoside (15922818)	−4.9364	GP	ANTHO
Procyanidin B2 3'-gallate (*122738)	−4.9230	GP	PROCY
(E)-resveratrol (445154)	−4.7791	P/GP	STYL
Malvidin-3-O-(6-acetyl)-glucoside-4-vinylphenol (*44257035)	−4.7603	GP	ANTHO
(E)-isoprenyl-p-coumarate (*637542)	−4.7592	P	PHE AC Ester
Delphinidin-3-O-(6-(Z)-p-coumaroyl)-glucoside (44256898)	−4.7251	GP	ANTHO
Malvidin-3-O-glucoside-4-vinylcatechol (44257036)	−4.7080	GP	ANTHO
Petunidin-3-O-glucoside-4-vinylphenol (*443651)	−4.6519	GP	ANTHO
Peonidin-3-O-(6-(E)-p-coumaroyl)-glucoside (443654)	−4.6496	GP	ANTHO
Vitisin B of malvidin-3-O-(6-p-coumaroyl)-glucoside (71308302)	−4.6318	GP	ANTHO
Malvidin-3-O-(6-(E)-p-coumaroyl)-glucoside-4-vinylphenol (*44256995)	−4.5634	GP	ANTHO
Quercetin-7-O-neohesperidoside (*5280343)	−4.5625	GP	FLAV Glyc
Peonidin-3-O-glucoside-4-vinylphenol (*443654)	−4.5593	GP	ANTHO
(E)-bencylcaffeate (5919576)	−4.5426	P	PHE AC
Petunidin-3-O-(6-(E)-p-coumaroyl)-glucoside (176449)	−4.5289	GP	ANTHO
Cyanidin-3-O-(6-(Z)-p-coumaroyl)-glucoside (5282067)	−4.4787	GP	ANTHO
(Z)-isoprenyl-p-coumarate (*637542)	−4.4233	GP	PHE AC Ester

Continued

Pyruvic derivative of delphinidin-3-O-glucoside (165558)	−4.3155	GP	ANTHO
Pyruvic derivative of peonidin-3-O-(6-acetyl)-glucoside	−4.3068	GP	ANTHO
Pyruvic derivative of petunidin-3-O-(6-p-coumaroyl)-glucoside (72193651)	−4.2970	GP	ANTHO
(Z)-caftaric acid (72551521)	−4.2795	GP	PHE AC
Delphinidin-3-O-(6-acetyl)-glucoside (15385440)	−4.2767	GP	ANTHO
Quercetin-3-O-galactoside (5281643)	−4.2540	GP	FLAV Glyc
Pyruvic derivative of peonidin-3-O-glucoside (443654)	−4.2522	GP	ANTHO
3.4-dihydroxyvinylbencene (151398)	−4.2318	P	STYL
Malvidin-3-O-glucoside-4-vinylphenol (44257035)	−4.2188	GP	ANTHO
(E)-caffeic acid (717531)	−4.2118	P	PHE AC
Pyruvic derivative of malvidin-3-O-(6-p-coumaroyl)-glucoside (72193651)	−4.2087	GP	ANTHO
Vitisin B of malvidin-3-O-(6-acetyl)-glucoside (*71308302)	−4.2065	GP	ANTHO
Apigenin (5280443)	−4.1989	P	FLAV
Methylgallate (7428)	−4.1983	GP	FLAV
Procyanidin B3 (4R-8 (+)C(-)C) (146798)	−4.1970	GP	PROCY
Delphinidin-3-O-glucoside-4-vinylphenol (*443650)	−4.1664	GP	ANTHO
Pyruvic derivative of delphinidin-3-O-(6-acetyl)-glucoside (15385440)	−4.1439	GP	ANTHO
(+)-gallocatechin (65084)	−4.1433	GP	CAT
Isorhamnetin-3-O-glucoside (5318645)	−4.1284	GP	FLAV Glyc
Procyanidin B8 (4R-6 (+)C(+)E) (130556)	−4.1111	GP	PROCY
Fisetin (5281614)	−4.1051	P	FLAV
Malvidin-3-O-(6-acetyl)-glucoside-4-vinylcatechol (*44257036)	−4.0565	GP	ANTHO
Peonidin-3-O-glucoside (443654)	−4.0496	GP	ANTHO
Oroxylin A (5320315)	−4.0489	GP	ANTHO
Petunidin-3-O-(6-acetyl)-glucoside (44256961)	−4.0434	GP	ANTHO
Naringin (442428)	−4.0359	P	FLAV
Malvidin-3-O-(6-(E)-caffeoyl)-glucoside (44256989)	−4.0332	GP	ANTHO

**Names, origin and structures were taken from Paulino *et al.* [24] with permission of all co-authors.