



Interactions of antioxidants isolated from tart cherry (*Prunus cerasus*) fruits

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ABSTRACT

Tart cherry produces various kinds of polyphenolics in its fruits that include cyanidin derivatives (mostly cyanidin 3-glucosylrutinoside, cyanidin 3-rutinoside, cyanidin sophoroside), peonidin 3-glucoside; kaempferol, quercetin, and isorhamnetin and their derivatives, as well as the alkaloid, melatonin. The antioxidant capacities of these constituents were analysed using the TEAC antioxidant assay. Kaempferol, quercetin, isorhamnetin 3-rutinoside, cyanidin 3-rutinoside, and melatonin showed significant antioxidant capacities; of these, kaempferol proved to be the most active. In order to determine how these constituents interact in terms of expression of their antioxidant action, we performed an isobolographic analysis. Using different dose ratios (e.g., 1:1, 1:2, 1:4) for the selected polyphenol constituents, we found evidence that three types of interactions may occur: synergistic, additive, and negative. The most important and new finding here was that pairs of compounds with the highest antioxidant capacity (e.g., kaempferol and melatonin at a dose ratio of 2:1, respectively, and cyanidin 3-rutinoside and isorhamnetin 3-rutinoside at a dose ratio of 1:4, respectively) showed strong synergistic types of interactions. Additive or negative types of interactions occurred for pairs of compounds that had lower antioxidant capacities. Thus, not all polyphenols in tart cherry fruits are equally effective in alleviating oxidative stress. Those which are most effective are likely to be acting synergistically.

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1. Introduction

The finding that tart cherry fruits contain significant levels of polyphenolics (anthocyanins and other flavonoids), as well as the alkaloid, melatonin, points to tart cherry being one of the more healthful edible berries (Kirakosyan, Seymour, Urcuyo Llanes, Kaufman, & Bolling, 2009). In general, these different metabolites in tart cherry fruits may possess vastly different antioxidant capacities. This would explain why they have different abilities to scavenge oxygen free radicals and other reactive species (ROX) that are so important in their action in reducing the oxidative stress and inflammation that exacerbate heart and other diseases in humans. In this context, the biological effectiveness of tart cherries may also be due to phytochemical interactions that accomplish synergistic effects. Thus, it is not surprising that whole tart cherry fruit products, or mixtures of tart cherry secondary metabolites, could be

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biologically more active than individual compounds (Seymour et al., 2008).

The basis for these differences in antioxidant capacities of the different polyphenol constituents and melatonin in tart cherry fruits could be explained by several kinds of interactions that may occur at target sites of action, namely, synergistic, additive, or inhibitory/antagonistic. One can show which of these types of interactions occurs for different pairs of constituents used in different dose ratios, using an isobolographic type of analysis (Wagner, 2006; Williamson, 2001).

Herbal supplements are commonly used in mixtures, also called cocktails, to prevent or to provide positive treatment for several diseases. For many years, the therapeutic effectiveness of such mixtures raises the bar when one compares them to a single compound and its therapeutic effect. The multi-constituent therapeutic concept, that constituted the first approach toward rationalising this phenomenon, is attributed to Berenbaum (1989). Simply put, he outlined synergy effects between two natural compounds, employing two mathematical equations, in which the effect of a drug combination is compared with single components. The first equation:

$$E(da, db) > 4E(da) + E(db)$$

shows that the total effect of a combination is greater than that expected from the sum of the effects of single components. In addition to this, the second equation assumes that synergy may exist if the effect of a combination is greater than that of each of the individual agents. It is denoted as: $E(da, db) > 4E(da)$ and $E(da, db) > 4E(db)$, where $E^{1/4}$ is the observed effect and da and db are the doses of agents a and b (Williamson, 2001, 2005).

In this study, our primary working hypothesis was that pairs of selected antioxidant constituents isolated from tart cherry fruits would manifest synergistic, additive or antagonistic types of interactions. By testing this hypothesis, the most important and new finding here is that pairs of compounds with the highest antioxidant capacity showed strong synergistic interactions, whereas pairs of compounds that had the lowest antioxidant capacities manifested additive or antagonistic interactions. The implications of these findings clearly point to which constituents in tart cherry fruits are the most active ones in alleviating oxidative stress and also shed light on possible mechanisms of action of these molecules at different target sites.

2. Materials and methods

2.1. Sources of chemicals

Solvents employed for extraction and LC–MS analysis were obtained from Fisher Scientific Co., Pittsburgh, PA. Quercetin, kaempferol, and melatonin were purchased from Sigma Chemical Co., St. Louis, MO. Anthocyanins (cyanidin 3-rutinoside, peonidin 3-glucoside, and pelargonidin) and the flavonoid, isorhamnetin 3-rutinoside, were obtained from Extrasynthèse (Genay, France) and Polyphenols Laboratories (Sandnes, Norway).

2.2. Extraction of anthocyanins and other metabolites

The powder from individually quick-frozen (IQF) tart cherries was kindly supplied by The Cherry Marketing Institute (CMI) (Lansing, MI, USA). One gram of powder was extracted with 10 ml methanol:water:acetic acid (85:15:0.5 v/v/v) for anthocyanins and 10 ml methanol:water (80:20 v/v) for other flavonoids and melatonin in 15-ml screw-cap tubes at 4 °C and placed on a gyratory shaker overnight in the dark. The samples were then vortexed and sonicated. After filtration through a 0.22- μ m filter, the extracts were ready for analysis.

2.3. Liquid chromatography–mass spectrometry (LC–MS) analysis of anthocyanins and other metabolites

An Alliance 2695 HPLC (Waters Corp., Milford, MA, USA) was used to generate a binary gradient with 0.05% TFA in water as the aqueous solvent (A) and 0.05% TFA in acetonitrile as the organic modifier (B). Chromatographic separation of anthocyanins and other flavonoids was achieved with a Gemini 5- μ m C18 150 \times 2 mm reverse phase column (Phenomenex, Torrance, CA, USA) held at 35 °C, using a flow rate of 0.19 ml/min. The column was initially equilibrated to 8% B, increased to 18% B over 10 min, 28% B over the next 8 min, 40% B in 1 min, 60% B in 3 min, then returned to the initial conditions. Effluent from the HPLC column was directed to the electrospray ionisation probe of a TSQ Quantum Ultra AM triple quadrupole mass spectrometer (Thermo-Finnigan, San Jose, CA, USA). Positive ions were generated with the following parameters: spray voltage 3000 V, sheath gas 40, aux gas 10, and capillary temperature 250 °C. Tube lens voltages and collision energies were optimised for each compound.

Data were collected in the centroid mode. Single reaction monitoring (SRM) was used for mass analysis and quantification.

Estimation of melatonin by HPLC electrospray mass spectrometry was carried out as reported previously (Kirakosyan et al., 2009).

2.4. Antioxidant assays

All standard samples (1 mg each) were individually dissolved in 10 ml methanol:water:acetic acid (85:15:0.5; v/v/v) for anthocyanins or methanol:water (80:20; v/v) for other metabolites and filtered through a 0.22- μ m filter. Total antioxidant capacity was based on TEAC (Trolox Equivalent Antioxidant Capacity) and was measured using The Cayman Chemical Antioxidant Assay Kit (Cayman Chemical, Ann Arbor, MI, USA), following the manufacturer's directions. Basically, we chose the TEAC method because it is more applicable and more useful for large-scale analyses involving different combinations of metabolites (Kirakosyan et al., 2003). ORAC (Oxygen Radical Antioxidant Capacity) is a more sensitive assay, which utilises fluorescence detection (Blando, Gerardi, & Nicoletti, 2004). The latter has been applied to small case studies of tart cherry antioxidants as well. However, no significant differences have been found between the two methods (data not shown).

2.5. Isobolographic analysis

In our study, we employed the isobolographic analysis method (Wagner, 2006; Williamson, 2001) to show the three kinds of interactions (synergy, additive or antagonistic/negative) that occur for different pairs of compounds at the relevant dose ratios (1:1 versus 1:2 versus 1:4). Fig. 1 graphically illustrates these three different types of interactions using this method. The isobol graphs were generated with the CombiTool program kindly provided by Dr. Jürgen Sühnel (Jena, Germany). Our analysis compares the effect differences between the Loewe additivity and experimental data.

2.6. Statistical analysis of data

Experiments were repeated at least three times, and the data were analysed statistically. All results are given as mean \pm standard deviation (SD).

3. Results

Cyanidin 3-rutinoside, peonidin 3-glucoside, pelargonidin, isorhamnetin 3-rutinoside, kaempferol, quercetin, and melatonin were detected in sour cherry fruits using LC–MS (Table 1). In the

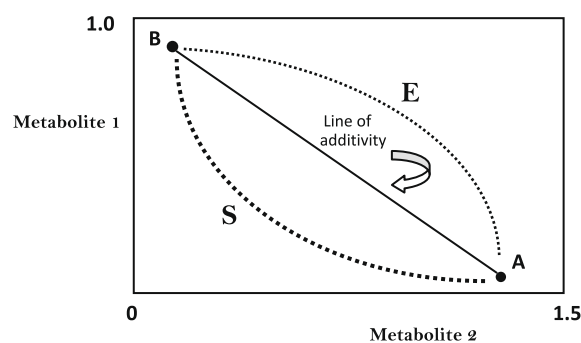


Fig. 1. Example of isobolograms showing the potential relationship of metabolites 1 and 2. Intercepts A and B represent doses of each constituent that individually provide. S represents a synergistic interaction. E represents an antagonistic interaction. The line connecting points A and B represents an additive effect.

Table 1

Comparison of levels of several anthocyanins, and other flavonoids, as well as melatonin, in sour cherry IQF (instant quick-frozen) powder. Data are expressed in $\mu\text{g/g}$ dry weight biomass (melatonin is expressed as ng/g dry weight biomass).

Cyanidin 3-rutinoside	226.1 \pm 44.2
Peonidin 3-glucoside	38.8 \pm 6.7
Pelargonidin	8.64 \pm 0.9
Isorhamnetin 3-rutinoside	176.6 \pm 52.2
Quercetin	292.6 \pm 56.5
Kaempferol	85.9 \pm 9.9
Melatonin	7.5 \pm 0.9

current study, we focused primarily on these compounds because of their relatively high antioxidant capacities, as reported previously (Kirakosyan et al., 2009). The anthocyanins and other flavonoids, as well as melatonin, found in tart cherry fruits have been reported to possess various phytotherapeutic activities that are based on their modes of action at different target sites (Jayaprakasam, Vareed, Olson, & Nair, 2005; Kim, Heo, Kim, Yang, & Lee, 2005). The respective antioxidant activities of these compounds, as determined by the TEAC method, are presented in Fig. 2. The antioxidant capacities of kaempferol and quercetin appear to be higher than those of the other metabolites. Interestingly, kaempferol proved to be the most active (4.5 mM TEAC), even though this molecule does not have the catechol system in the B ring, the most important feature for antioxidant ability (Li, Liu, Zhang, & Yu, 2008).

The antioxidant capacities of a combination of two sets of metabolites at relevant dose ratios were also examined, in order to analyse their potential synergistic, additive or antagonistic effects. The experimental parameters have been identified using the TEAC antioxidant capacity test for different metabolite combinations at relevant dose ratios (1:1 versus 1:2 versus 1:4) for two agents (Table 2). The results in Table 2 show that mixtures of several two-compound combinations at relevant dose ratios may have possible synergistic, additive, or antagonistic effects.

Having such experimental parameters allows one to explore more appropriate approaches to express the nature of these combination effects. Isobolograms were therefore generated as described in Section 2. The results of the isobolographic analysis for several combinations of constituents are presented in Figs. 3–6 where the median interaction indices are presented for all compounds for each fixed-ratio combination. For example, a combination of melatonin and quercetin at ratio of 1:4 shows an antioxidant activity of 5.058 mM TEAC, which is more than the expected additive effect (Table 2). For this interaction, we have

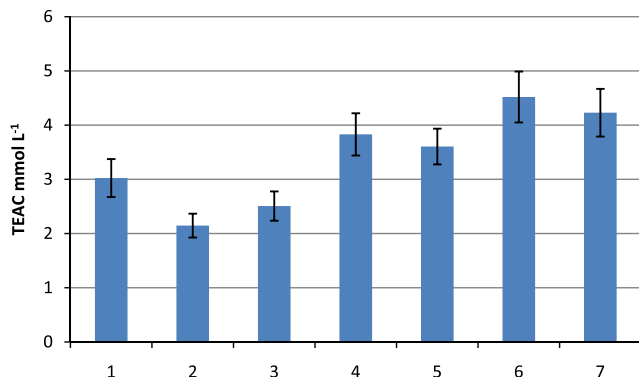


Fig. 2. Antioxidant capacity of selected metabolites. Numbers along the abscissa refer to the following: 1, cyanidin 3-rutinoside; 2, peonidin 3-glucoside; 3, pelargonidin; 4, melatonin; 5, isorhamnetin 3-rutinoside; 6, kaempferol; 7, quercetin. Values presented are mean \pm SD; $n = 3$.

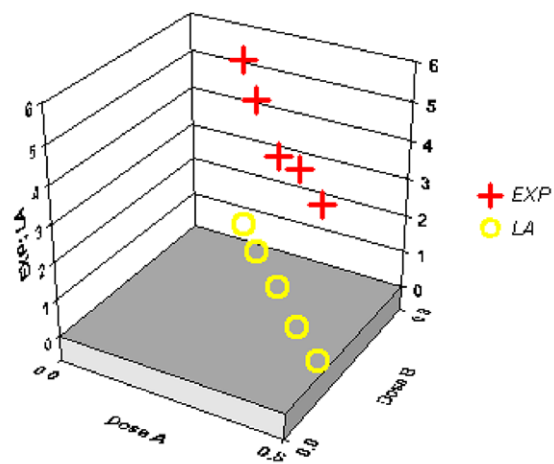
Table 2

The antioxidant capacities (TEAC) of a combination of two sets of metabolites at relevant dose ratios (1:1 versus 1:2 versus 1:4).

Interacting compounds	Dose ratio	TEAC (mM)
Cyanidin 3-rutinoside:melatonin	1:1	1.60 \pm 0.223
Cyanidin 3-rutinoside:melatonin	2:1	1.97 \pm 0.254
Cyanidin 3-rutinoside:melatonin	4:1	2.44 \pm 0.262
Cyanidin 3-rutinoside:melatonin	1:2	1.83 \pm 0.198
Cyanidin 3-rutinoside:melatonin	1:4	3.73 \pm 0.392
Cyanidin 3-rutinoside:kaempferol	1:1	1.41 \pm 0.182
Cyanidin 3-rutinoside:kaempferol	2:1	1.53 \pm 0.177
Cyanidin 3-rutinoside:kaempferol	4:1	2.02 \pm 0.253
Cyanidin 3-rutinoside:kaempferol	1:2	1.13 \pm 0.155
Cyanidin 3-rutinoside:kaempferol	1:4	2.23 \pm 0.249
Cyanidin 3-rutinoside:isorhamnetin-3-rutinoside	1:1	4.15 \pm 0.511
Cyanidin 3-rutinoside:isorhamnetin-3-rutinoside	1:2	3.97 \pm 0.433
Cyanidin 3-rutinoside:isorhamnetin-3-rutinoside	2:1	3.31 \pm 0.367
Cyanidin 3-rutinoside:isorhamnetin-3-rutinoside	1:4	4.20 \pm 0.473
Cyanidin 3-rutinoside:isorhamnetin-3-rutinoside	4:1	2.70 \pm 0.312
Pelargonidin:melatonin	1:1	2.26 \pm 0.228
Pelargonidin:melatonin	2:1	2.40 \pm 0.285
Pelargonidin:melatonin	4:1	3.11 \pm 0.355
Pelargonidin:melatonin	1:2	0.602 \pm 0.072
Pelargonidin:melatonin	2:1	1.28 \pm 0.167
Pelargonidin:kaempferol	1:1	1.79 \pm 0.222
Pelargonidin:kaempferol	2:1	2.01 \pm 0.276
Pelargonidin:kaempferol	4:1	1.27 \pm 0.167
Pelargonidin:kaempferol	1:2	0.591 \pm 0.082
Pelargonidin:kaempferol	1:4	2.16 \pm 0.277
Kaempferol:melatonin	1:1	5.22 \pm 0.456
Kaempferol:melatonin	2:1	5.42 \pm 0.387
Kaempferol:melatonin	4:1	5.29 \pm 0.465
Kaempferol:melatonin	1:2	5.00 \pm 0.398
Kaempferol:melatonin	1:4	4.81 \pm 0.521
Cyanidin 3-rutinoside:peonidin 3-glucoside	1:1	1.92 \pm 0.232
Cyanidin 3-rutinoside:peonidin 3-glucoside	2:1	2.21 \pm 0.199
Cyanidin 3-rutinoside:peonidin 3-glucoside	4:1	1.72 \pm 0.211
Cyanidin 3-rutinoside:peonidin 3-glucoside	1:2	2.11 \pm 0.255
Cyanidin 3-rutinoside:peonidin 3-glucoside	1:4	1.92 \pm 0.168
Peonidin 3-glucoside:isorhamnetin 3-rutinoside	1:1	2.15 \pm 0.276
Peonidin 3-glucoside:isorhamnetin 3-rutinoside	2:1	2.33 \pm 0.199
Peonidin 3-glucoside:isorhamnetin 3-rutinoside	4:1	1.80 \pm 0.167
Peonidin 3-glucoside:isorhamnetin 3-rutinoside	1:2	1.50 \pm 0.202
Peonidin 3-glucoside:isorhamnetin 3-rutinoside	1:4	2.39 \pm 0.199
Peonidin 3-glucoside:quercetin	1:1	1.64 \pm 0.212
Peonidin 3-glucoside:quercetin	2:1	2.24 \pm 0.311
Peonidin 3-glucoside:quercetin	4:1	2.06 \pm 0.199
Peonidin 3-glucoside:quercetin	1:2	1.71 \pm 0.155
Peonidin 3-glucoside:quercetin	1:4	3.42 \pm 0.298
Melatonin:quercetin	1:1	4.02 \pm 0.378
Melatonin:quercetin	2:1	4.57 \pm 0.488
Melatonin:quercetin	4:1	4.45 \pm 0.473
Melatonin:quercetin	1:2	4.67 \pm 0.513
Melatonin:quercetin	1:4	5.06 \pm 0.488
Isorhamnetin 3-rutinoside:quercetin	1:1	4.69 \pm 0.505
Isorhamnetin 3-rutinoside:quercetin	2:1	4.47 \pm 0.486
Isorhamnetin 3-rutinoside:quercetin	4:1	3.89 \pm 0.344
Isorhamnetin 3-rutinoside:quercetin	1:2	4.23 \pm 0.399
Isorhamnetin 3-rutinoside:quercetin	1:4	4.82 \pm 0.503
Peonidin 3-glucoside:melatonin	1:1	1.22 \pm 0.155
Peonidin 3-glucoside:melatonin	2:1	1.97 \pm 0.222
Peonidin 3-glucoside:melatonin	4:1	1.93 \pm 0.202
Peonidin 3-glucoside:melatonin	1:2	1.37 \pm 0.155
Peonidin 3-glucoside:melatonin	1:4	2.44 \pm 0.257
Cyanidin 3-rutinoside:quercetin	1:1	1.09 \pm 0.091
Cyanidin 3-rutinoside:quercetin	2:1	1.74 \pm 0.198
Cyanidin 3-rutinoside:quercetin	4:1	1.94 \pm 0.244
Cyanidin 3-rutinoside:quercetin	1:2	1.37 \pm 0.142
Cyanidin 3-rutinoside:quercetin	1:4	2.91 \pm 0.315
Kaempferol:quercetin	1:1	3.43 \pm 0.325
Kaempferol:quercetin	2:1	3.51 \pm 0.414
Kaempferol:quercetin	4:1	3.46 \pm 0.333
Kaempferol:quercetin	1:2	3.52 \pm 0.328
Kaempferol:quercetin	1:4	3.51 \pm 0.379

Table 2 (continued)

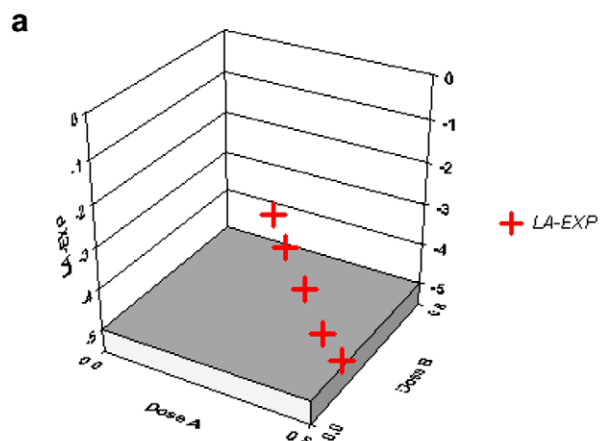
Interacting compounds	Dose ratio	TEAC (mM)
Kaempferol:isorhamnetin 3-rutinoside	1:1	3.33 ± 0.367
Kaempferol:isorhamnetin 3-rutinoside	2:1	3.31 ± 0.298
Kaempferol:isorhamnetin 3-rutinoside	4:1	3.35 ± 0.295
Kaempferol:isorhamnetin 3-rutinoside	1:2	3.36 ± 0.316
Kaempferol:isorhamnetin 3-rutinoside	1:4	3.21 ± 0.354
Kaempferol:peonidin 3-glucoside	1:1	1.51 ± 0.172
Kaempferol:peonidin 3-glucoside	2:1	1.54 ± 0.165
Kaempferol:peonidin 3-glucoside	4:1	1.32 ± 0.098
Kaempferol:peonidin 3-glucoside	1:2	1.04 ± 0.111
Kaempferol:peonidin 3-glucoside	1:4	1.20 ± 0.134
Quercetin:pelargonidin	1:1	1.48 ± 0.175
Quercetin:pelargonidin	2:1	1.88 ± 0.221
Quercetin:pelargonidin	4:1	1.92 ± 0.235
Quercetin:pelargonidin	1:2	1.78 ± 0.192
Quercetin:pelargonidin	1:4	1.30 ± 0.122
Isorhamnetin 3-rutinoside:pelargonidin	1:1	0.941 ± 0.121
Isorhamnetin 3-rutinoside:pelargonidin	2:1	1.24 ± 0.152
Isorhamnetin 3-rutinoside:pelargonidin	4:1	1.22 ± 0.144
Isorhamnetin 3-rutinoside:pelargonidin	1:2	0.916 ± 0.078
Isorhamnetin 3-rutinoside:pelargonidin	1:4	0.812 ± 0.067
Peonidin 3-glucoside:pelargonidin	1:1	0.542 ± 0.039
Peonidin 3-glucoside:pelargonidin	2:1	0.192 ± 0.023
Peonidin 3-glucoside:pelargonidin	4:1	0.229 ± 0.035
Peonidin 3-glucoside:pelargonidin	1:2	0.194 ± 0.232
Peonidin 3-glucoside:pelargonidin	1:4	0.263 ± 0.199



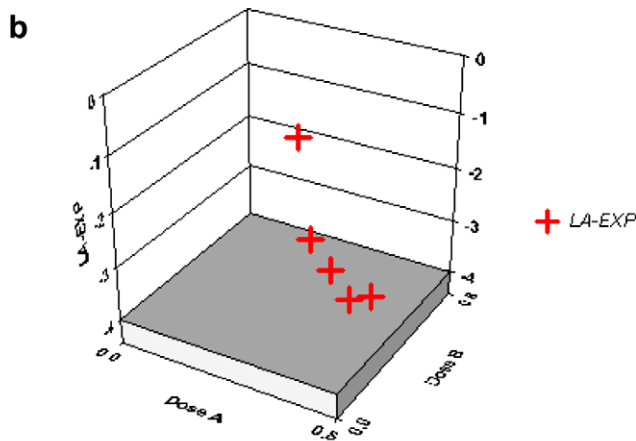
Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;

Fig. 3. Comparison of the Loewe additivity and experimental data combination effects for melatonin and quercetin.

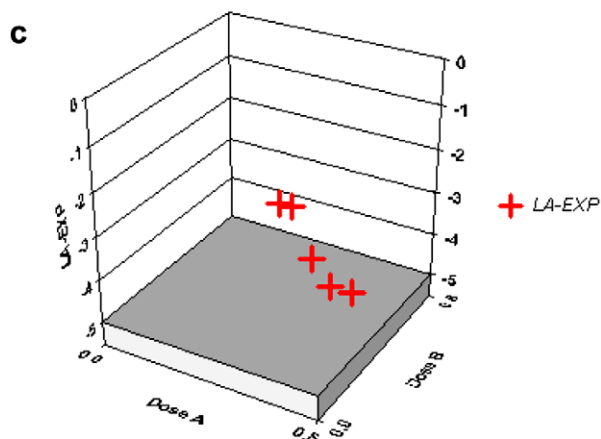
compared and presented the Loewe additivity combination effect with experimental data combination effect. This comparison showed strong synergistic interaction, as presented in Fig. 3. A



Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;

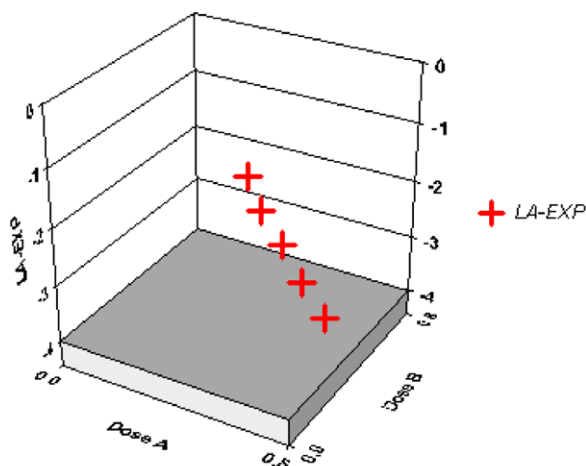


Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;



Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;

Fig. 4. The synergistic interactions of kaempferol and melatonin (a), cyanidin 3-rutinoside and isorhamnetin 3-rutinoside (b), and isorhamnetin 3-rutinoside and quercetin (c), based on the effect difference between the Loewe additivity and experimental data.



Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;

Fig. 5. The additive interaction of kaempferol and isorhamnetin 3-rutinoside, based on the effect difference between the Loewe additivity and experimental data.

similar synergistic effect based on the effect difference between the Loewe additivity and experimental data (Fig. 4a) is observed for the kaempferol and melatonin combination in a dose ratio of 2:1, with an antioxidant activity of 5.424 mM TEAC (Table 2).

Cyanidin 3-rutinoside and isorhamnetin 3-rutinoside, or isorhamnetin 3-rutinoside and quercetin at 1:4 ratios, show antioxidant activities of 4.198 and 4.822 mM TEAC, respectively (Table 2). These compound combinations are certainly candidates for synergistic interaction at the target site (Fig. 4b and c). Other results show an additive interaction, as depicted in Fig. 5 for kaempferol and isorhamnetin 3-rutinoside.

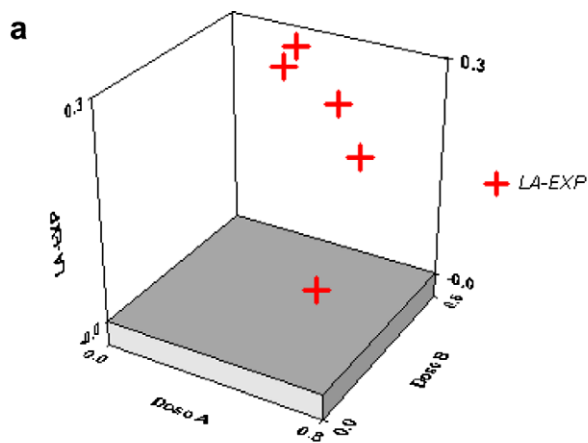
Several other combinations of compounds show antagonistic interactions, as can be seen for cyanidin 3-rutinoside and melatonin, cyanidin 3-rutinoside and kaempferol, cyanidin 3-rutinoside and peonidin 3-glucoside, pelargonidin and melatonin, pelargonidin and kaempferol, peonidin 3-glucoside and isorhamnetin 3-rutinoside, peonidin 3-glucoside and quercetin, peonidin 3-glucoside and melatonin, peonidin 3-glucoside and pelargonidin, kaempferol and quercetin, kaempferol and peonidin 3-glucoside, quercetin and pelargonidin, isorhamnetin 3-rutinoside and pelargonidin in all selected ratios. Two examples of these antagonistic interactions are shown in Fig. 6a and b.

Results of this study identify the primary sour cherry antioxidants that contribute to the observed pharmacological effects, and they point to which ones may act synergistically, additively, or negatively. Taken together, our results show that the overall pattern of the compounds with strong antioxidant capacities show interactions that are primarily synergistic.

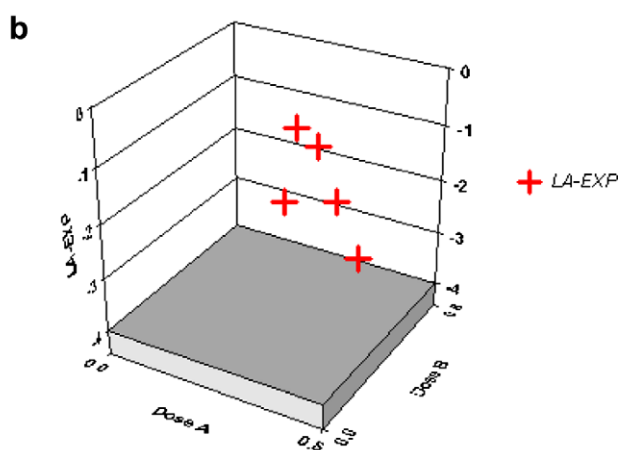
4. Discussion

The interaction of secondary metabolites in intact plants may play a crucial role for organisms in response to environmental and biotic stresses. In such situations, even very small amounts of a particular metabolite may act as a signalling molecule to trigger several biochemical reactions. Several examples are well discussed and highlighted (Cseke et al., 2006; Heil & Ton, 2008; Kirakosyan et al., 2003). In some cases, one metabolite may alter permeability or transport of a second one in order to be active within a cell. Sometimes, cellular membranes can serve as target sites for metabolite interactions. Such interactions may influence receptor proteins in the membranes, especially the extent of protein–protein or protein–ligand binding. In the case of flavonoids, they probably affect membrane lipid viscosity or protein movement (Tarahovsky, Muzafarov, & Kim, 2008). The interaction of plant secondary metabolites in intact plants and their action in vital functions of organisms raise the bar for synergistic effects of metabolites *in vitro* at the target site. This idea has been adopted by pharmacologists, in order to explore combinations of several metabolites in multi-target therapy (Ulrich-Merzenich, Zeitler, Vetter, & Kraft, 2009). The importance of affecting multiple targets could be beneficial when dealing with complex diseases, such as cancer, chronic inflammation, chronic viral infection, and many others (Frantz, 2005; Roth, Sheffler, & Kroeze, 2004; Tortora, Bianco, & Daniele, 2004; Zimmermann, Lehár, & Keith, 2007).

Synergistic interactions indicate that the compounds in the mixture act via different mechanisms and/or on different disease-associated targets. Yet, the combined bioactivity of the mixture is potentiated when the interaction results in improved solubility, absorption, safety, stability, or bioavailability of the active principles (Boik, Newman, & Boik, 2008; Freeman & Spelman, 2008; Greco, Bravo, & Parsons, 1995; Liu, 2003; Spinella, 2002). Negative interactions (interferences) may also occur when certain components of the mixture inhibit the full biological activity of pharmacologically-active compounds by reducing their stability or bioavailability or by enhancing their metabolism.



Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;



Median Effect Function: $aA=0.10399$ $aB=0.10399$, $mA=0.006324$ $mB=0.006324$;

Fig. 6. The antagonistic interactions of peonidin 3-glucoside and pelargonidin (a) and cyanidin 3-rutinoside and melatonin (b), based on the effect difference between the Loewe additivity and experimental data.

Several plant extracts have been shown to exhibit synergistic activity against microorganisms. Hemaiswarya, Kruthiventi, and Doble (2008) describe in detail the observed synergy and mechanism of action between natural products, including flavonoids and essential oils and synthetic drugs, in effectively combatting bacterial, fungal, and mycobacterial infections. They have reported that the mode of action of such combinations differs significantly from that of the same drugs acting individually. Lansky et al. (2005) showed that possible synergistic action occurs in connection with prostate cancer suppression by anatomically discrete pomegranate fractions, which are made up of a combination of several polyphenols and seed oils.

The current studies with tart cherry fruit metabolites reveal several important mechanistic concepts that may also hold true for edible fruits of other plants. These include the following: (1) not all of the polyphenolic constituents identified in the ripe fruits are equally effective as antioxidants; (2) when used in combination at relevant doses, some pairs of metabolites manifest synergistic interactions, others additive interactions, and still others, antagonistic interactions *vis-a-vis* antioxidant activity. Hence, one cannot generalise that all antioxidant interactions between metabolites are likely to be synergistic. However, the most significant finding from our study is that compounds with strong antioxidant capacities show interactions that are primarily synergistic. Such synergistic interactions may be explained by enhanced uptake, absorption, metabolism and reduced excretion (pharmacokinetics) or by enhanced effectiveness (binding to receptor molecules like bioactive proteins that enhance protein–protein or protein–ligand interactions) at the target site(s) of action (pharmacodynamics).

Future studies must be well-justified, well-designed, and interpreted properly, in order to be suitable for medical applications. In this regard, several factors and criteria must be considered that may affect the expected results. For example, the interaction of hydrophilic and hydrophobic compounds in medicinal plant extracts, as regards both permeability and metabolism of the compounds in a mixture, may be altered. Additionally, in conventional pharmacology, mixtures of pharmacologically-active compounds are generally thought to induce problematic pharmacokinetics, as well as toxicity (Wermuth, 2004).

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