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Protection of human erythrocytes against oxidative stress by berries[1]

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Abstract. Berries contain several bioactive compounds that can protect against oxidative stress. In this study we evaluated the protective effect of different sequential extracts (ethyl acetate, ethanol and water) of seven berry species: bilberry (*Vaccinium myrtillus*), blackcurrant (*Ribes nigrum*), elderberry (*Sambucus nigra*), lingonberry (*Vaccinium vitis-idaea*), rose hips (*Rosa sp.*), sea buckthorn (*Hippohae rhamnoides*) and strawberry (*Fragaria* × *ananassa*). The protective effect was tested on human erythrocytes and the antioxidant capacity was also evaluated *in vitro* by the FRAP assay. In the erythrocyte assay all sea buckthorn extracts were superior in antioxidant effect to other berry extracts. The ethyl acetate extract of bilberries, and the ethanol and water extracts of blackcurrants, also protected the erythrocytes from oxidation. In contrast, water extracts of rose hips, bilberries and strawberries had a pro-oxidant effect on erythrocytes. The water extract of rose hips was superior to the other berry extracts in the FRAP assay. Thus, the results of the erythrocyte assay did not correlate with the results of the FRAP assay, but provided additional insights into the potential protective effects of berry extracts against oxidative stress.

Keywords: Antioxidants, FRAP, polyphenols, red blood cells, SagM

1. Introduction

Epidemiological studies suggest that diets rich in fruit and vegetables promote health and delay the onset of several diseases associated with oxidative stress [1]. Berries contain numerous nutrients such as dietary fibres, vitamins, minerals, carotenoids and tocopherols as well as other beneficial phytochemicals, especially polyphenols [2]. All polyphenols are free radical scavengers and they participate in the regeneration of other antioxidants and may protect cell constituents from oxidative stress [2]. Different berry species have different profiles of polyphenols, which may confer different antioxidative benefits. Polyphenol levels, such as anthocyanins, are high in blackcurrants (*Ribes nigrum*), and consist predominantly of glucosylated and rutinosylated cyanidin and delphinidin [3]. Bilberries (*Vaccinium myrtillus*) and elderberries (*Sambucus nigra*) also have a high content of anthocyanins [4, 5]. Bilberries are of great interest in this respect, since they contain very diverse and complex mixtures of anthocyanins (up to fifteen congeners) derived from at least five anthocyanidins. In addition bilberries contain a rare type of procyanidin (A-type), associated with antibacterial effects, and previously found in high amounts in for example cranberries [6]. The content of flavonols is thought in general to be lower in bilberries than in lingonberries (*Vaccinium vitis-idaea*) [3]. Lingonberries are, however, rich in resveratrol, an important phenolic compound of the stilbene group [7].

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Resveratrol is present in similar amounts as in grapes [8], possibly contributing to health benefits of red wine. Sea buckthorn berries (*Hippophae rhamnoides*) and rose hips (*Rosa sp.*) are rich in proanthocyanidins [9, 10]. In addition to different polyphenols both sea buckthorn and rose hips are significant sources of ascorbic acid and several other bioactive compounds [11–13]. Different *Rubus* species contain high amounts of ellagitannins and strawberries (*Fragaria* × *ananassa*) contain for example 15 times more ellagic acid than other fruits and berries [14].

Chemical analyses have previously established the high antioxidant activity *in vitro* of many berry species and mostly attributed the effect to the content of polyphenols and ascorbic acid (and in some berry species also to carotenoids and tocopherols) [15–18], although the relevance of these results to humans is still not sufficiently studied. It has been argued that the direct antioxidant activity of polyphenols *in vivo* is probably limited due to low concentrations (except in the gastrointestinal tract) and instead, a pro-oxidant effect of polyphenols involving activation of antioxidant enzymes has been proposed among several other mechanisms of action [19]. Whilst clinical studies are the ultimate way to study the effects of a complex diet on oxidative stress, a cellular antioxidant assay using erythrocytes has been developed which, alongside chemical assays, may be useful for early screening purposes. In humans, erythrocytes play a critical role in antioxidant protection of the blood, using several intracellular mechanisms to protect the integrity and functionality of haemoglobin against oxidative stress [20–22]. The erythrocytes are uniform, anucleate, easily accessible and lack the capacity for protein synthesis, thus limiting their responses to biochemical stimuli to certain defined enzymatic processes, which simplifies data interpretation. Moreover, they are anaerobic cells that lack mitochondria, which limit their internal production of free radicals.

In this study the protective effects of sequential extracts of seven berry species against oxidative stress were compared in an erythrocytic assay as well as in an iron reduction assay (FRAP) [23] commonly used to study antioxidant capacity *in vitro*.

2. Materials and methods

2.1. Chemicals

Heptane, ethyl acetate and ethanol were obtained from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO), catechin and H_2O_2 were purchased from Sigma-Aldrich (Seelze, Germany). Saline-adenine-glucose-mannitol medium (SagM) was provided by Macopharma (Helsingborg, Sweden).

2.2. Plant material

For this study berries were sampled at full maturity. Depending on berry species approximately 50–250 g of berries was harvested by hand from multiple places on the plants, avoiding unripe or over-ripe berries. Blackcurrants (*Ribes nigrum* 'Jadrenaja'), rose hips (*Rosa* 'BRo 484'), sea buckthorn berries (*Hippohae rhamnoides* 'Gibrid Pertjik') and strawberries (*Fragaria* × *ananassa* 'BFr 0121') were collected from the germplasm collection at the Swedish University of Agricultural Sciences, Balsgård. Additional berry samples were collected in the wild: bilberries (*Vaccinium myrtillus*), elderberries (*Sambucus nigra*) and lingonberries (*Vaccinium vitis-idaea*). Approximately 50 g fresh fruits from each species (the seeds in rose hips and sea buckthorn were removed) were lyophilized and thereafter milled to a fine powder in a laboratory mill (Yellow line, A10; IKA-Werke, Staufen, Germany) before extraction.

2.3. Preparation of different berry extracts

A sequential extraction was performed using 1) heptane, 2) ethyl acetate, 3) ethanol and 4) distilled water starting from 1.25 g berry powder. For each extraction 25 mL solvent was used. The extracts were kept in an ultrasonic bath for 3 × 5 min before centrifugation at 4500 rpm for 10 min and the supernatant was collected. The solvents (heptane, ethyl acetate and ethanol, respectively) were then removed in a rotary evaporator with the temperature held below 30°C. The remaining dry extracts were dissolved in 1.265 mL of DMSO, then diluted 133 times in SagM, and stored at −20°C until analysis.

The water extracts were freeze-dried. Vacuum was applied at <0.2 mbar and the condenser temperature was set at −70°C during all the freeze-drying process. The initial temperature of the sample tray was −5°C, and after 16 h a temperature gradient was applied from −35°C to 10°C. The temperature was then maintained at 10°C until the extracts were completely dry. The dry extracts were dissolved in 12.65 mL SagM solution and then diluted 13.3 times in SagM solution before storage in freezer at −20°C.

2.4. Preparation of erythrocytes

A healthy human volunteer served as blood donor for erythrocytes. Peripheral venous blood samples were drawn into sodium K_2-EDTA vials (BD Vacutainer, UK). The vials were then centrifuged for 5 min at 2400 rpm. Plasma and leukocytes were removed and the erythrocytes were harvested by pipettes and transferred into new vials. The erythrocytes were washed twice with SagM. From the remaining packed erythrocytes, 0.12 mL was added to 12 mL of SagM. The previously obtained ethyl acetate, ethanol and water extracts were diluted (1 : 3) with SagM and mixed with the erythrocytes (1/3 extract and 2/3 purified erythrocytes). The erythrocyte suspension was incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 60 min. The erythrocytes were then washed twice in SagM and any extracellular potential micronutrients were thereby removed.

2.5. Analysis of protection against oxidative stress using erythrocytes

The cell pellet was lysed through the addition of dH_2O and the sample treated with the fluorescent dye 5-(and-6)-carboxy-2′,7′-dichlorofluorescein (DCF-DA), which becomes fluorescent as a result of oxidative damage. The sample was exposed to free radicals by addition of 167 mM hydrogen peroxide (hydroxyl free radical generator). The degree of damage was recorded after 10 min by measuring the fluorescence intensity of each sample. The mean DCF-DA fluorescence intensity was compared between triplicate samples of untreated erythrocytes (negative controls), hydrogen peroxide treated erythrocytes (positive controls) and erythrocytes pre-treated with berry extracts on three separate plates.

2.6. Analysis of FRAP antioxidant capacity

The antioxidant capacity was measured according to the FRAP (Ferric Reducing Ability of Plasma) assay developed by Benzie and Strain [23], but modified to fit a 96-well format [24]. Ten μL of the extracts were incubated at 37°C and then mixed with 260 μL ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6; 10 mM of 2,4,6-tripyridyl-s-triazine in 40 mM HCl; and 20 mM $FeCl_3$ in the ratio of 4 : 1 : 1; the solution was kept at 37°C). The absorbance was measured at 595 nm after 4 min on a plate reader (Sunrise, Tecan Nordic AB, Sweden). Fe^{2+} was used as a standard and L-ascorbic acid was used as a control where one mole of ascorbic acid corresponds approximately to two moles of FRAP.

2.7. Analyses of total phenols

The content of total phenols was measured using the Folin-Ciocalteu method [25]. In brief, five μL of the different extracts were mixed with 100 μL 5% ethanol, 200 μL Folin-Ciocalteu reagent, 2 mL of 15% Na_2CO_3 and 1 mL of dH_2O. The absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid was used as a standard and the total content of phenols was expressed as mg gallic acid equivalents/g dry weight (dw).

2.8. Analysis of ascorbic acid

The content of ascorbic acid (vitamin C) of the samples was determined on a Shimadzu HPLC system (SIL-10A autosampler, SCL-10AVP control unit, LC-10AD pump, SPD-10AV VP UV-Vis detector unit, BergmanLabora, Sweden). Each extract was diluted 10–100 fold with 2% meta-phosphoric acid to preserve the content of ascorbic acid. The isocratic eluent consisted of 0.05 M NaH_2PO_4 and ortho-phosphoric acid (8.5%), and pH of the eluent was adjusted to 2.8. The separation was performed using a Restek, 150 × 4.6 mm, column kept at 30°C (Column Chiller,

Sorbent AB) and a guard column. Detection was carried out at 254 nm and the flow was adjusted to 1.0 mL min^{-1}. Evaluation of data was done with Shimadzu Class-VP software (version 6.13 SP2) using retention times and spectral data as compared with an external standard of ascorbic acid. Each sample was analyzed in duplicate.

2.9. Statistical analysis

We used the software Minitab version 16 (Minitab, State College, PA, USA) to perform analyses of variance, and Tukey's *post hoc* test ($p = 0.05$) was used to reveal any significant differences between means. We also calculated Pearson correlation coefficients between the results of different assays.

3. Results

An erythrocytic biological assay, as well as a chemical ferric reduction assay (FRAP), was used to investigate the protection against oxidative stress from extracts of different berry species. The berry extract concentration used in the study was 1.86 mg/mL (or 0.62 mg/mL when incubating with erythrocytes), as initial studies showed full protection in the erythrocyte assay when incubating the cells with higher concentrations of some of the extracts. No cellular lysing was observed using this concentration.

3.1. Antioxidant capacity

The antioxidant capacity results obtained by the FRAP assay of the different extracts and samples are provided in Table 1. The water extracts yielded the highest antioxidant capacity among the different solvents. Using water as solvent, rose hip extract had the highest individual antioxidant capacity (948 mmol Fe^{2+}/g dw), which was approximately three-fold that of elderberry (260 mmol Fe^{2+}/g dw), and six-fold higher than blackcurrants (148 mmol Fe^{2+}/g dw). The antioxidant capacity was low both of the ethyl acetate and ethanol extracts, with the exceptions of bilberry ethyl acetate extract (249 mmol Fe^{2+}/g dw) and the rose hip ethanol extract (211 mmol Fe^{2+}/g dw).

3.2. Total phenol analyses

The phenolic contents of the extracts, using the Folin-Ciocalteu assay, are given in Table 1. The ethyl acetate extracts had the lowest content of total phenols with the exception of the bilberry extract, which contained 10.0 mg GAE/g dw. In the ethanol extracts, low amounts of phenolic compounds were detected for all berry species apart from the rose hip sample that contained 8.9 mg GAE/g dw. The highest amounts were, however, found in the water extracts, where rose hips had 36.5 mg GAE/g dw and elderberry had 11.2 mg GAE/g dw.

3.3. Ascorbic acid analyses

The ascorbic acid content of the samples is shown in Table 1. In ethyl acetate extracts of sea buckthorn and bilberry trace amounts of ascorbic acid were found (0.2 and 0.1 mg/g dw, respectively), whereas the amounts were below the level of detection for other berries. Sea buckthorn, blackcurrant and strawberry ethanol and water extracts also contained minor amounts of ascorbic acid. The rose hip ethanol and water extracts were however notably rich in ascorbic acid. The content in the ethanol extract was 10.7 mg/g dw and in the water extract 38.4 mg/g dw.

3.4. Protection of erythrocytes against oxidative stress

The heptane extract showed no effect in the erythrocyte model (results not shown). The level of protection of the other extracts is presented in Table 2. Sea buckthorn berries showed a superior antioxidant effect to that of all the other berries in all three extracts (from 52 to 67%). Bilberries showed high protection in the ethyl acetate extract (42%), whilst the ethanol and water extracts of blackcurrants also protected the erythrocytes from oxidation (37

Table 1
Content of ascorbic acid, total phenols and antioxidant capacity (Total FRAP) of sequential ethyl acetate, ethanol and water extracts, presented as mean (and pooled standard deviation, StDev) per dry weight (dw) of the plant material. (GAE = gallic acid equivalents)

Solvent/ Plant material	Ascorbic acid (mg/g dw)	Total phenols (mg GAE/g dw)	Total FRAP (mmol Fe^{2+}/g dw)
Ethyl acetate			
Bilberry	0.1	10.0	249.3
Blackcurrant	0.0	0.2	1.7
Elderberry	0.0	0.3	4.7
Lingonberry	0.0	0.3	2.1
Rose hip	0.0	0.6	5.5
Sea buckthorn	0.2	0.4	3.0
Strawberry	0.0	0.1	3.4
Pooled StDev	0.0	0.2	4.9
Ethanol			
Bilberry	0.0	0.3	0.8
Blackcurrant	1.3	2.2	29.3
Elderberry	0.0	2.9	37.3
Lingonberry	0.0	3.1	44.5
Rose hip	10.7	8.9	211.2
Sea buckthorn	1.4	1.2	22.9
Strawberry	0.6	1.5	26.7
Pooled StDev	0.0	0.1	1.1
Water			
Bilberry	0.0	4.8	99.2
Blackcurrant	2.8	6.2	148.0
Elderberry	0.0	11.2	259.5
Lingonberry	0.0	5.8	82.3
Rose hip	38.4	36.5	947.6
Sea buckthorn	2.7	1.8	47.1
Strawberry	0.0	3.3	64.0
Pooled StDev	0.0	0.6	5.4

and 50% respectively). In contrast, all rose hip extracts and the water extract of bilberries and strawberries showed pro-oxidant activity.

3.5. Correlation analyses

We could not find any significant correlation between the protective effects of berry extracts on erythrocytes on the one hand, and total antioxidant capacity on the other hand, with one exception: For ethyl acetate extracts the content of ascorbic acid (and its corresponding antioxidant capacity) was significantly ($p < 0.003$) correlated (Pearson correlation coefficient = 0.919) with erythrocyte protection. Furthermore, for ethyl acetate, ethanol and water extracts the correlation between total antioxidant activity and total content of polyphenols was very high (0.984–0.999, $p < 0.000$). For ethanol and water extracts, the correlation was also very high between total antioxidant activity and total content of ascorbic acid (0.968, $p < 0.000$), as well as between total content of ascorbic acid and total content of phenols (0.916–0.958, $p < 0.004$).

Table 2
Erythrocyte protection, antioxidant capacity (Total FRAP), contribution from ascorbic acid to antioxidant capacity (Ascorbate FRAP) and content of total phenols of sequential ethyl acetate, ethanol and water extracts of seven berry species presented as mean and pooled standard deviation (StDev). (GAE = gallic acid equivalents)

Solvent/ Plant material	Erythrocyte protection (%)	Total FRAP (μmol Fe^{2+}/L)	Ascorbate FRAP (μmol Fe^{2+}/L)	Total phenols (mg GAE/L)
Ethyl acetate				
Bilberry	42.4	463.1	1.6	18.6
Black currant	3.3	3.2	0.2	0.3
Elderberry	−2.3	8.7	0.0	0.6
Lingonberry	−0.5	3.9	0.0	0.6
Rose hip	−6.1	10.2	0.1	1.1
Sea buckthorn	51.9	5.5	3.6	0.7
Strawberry	11.7	6.3	0.0	0.2
Pooled StDev	4.9	9.0	0.1	0.3
Ethanol				
Bilberry	3.4	16	0.0	0.5
Black currant	36.6	54.3	28.9	4.0
Elderberry	21.0	69.3	0.7	5.4
Lingonberry	3.1	82.7	0.7	5.8
Rose hip	−14.8	392.2	230.7	16.5
Sea buckthorn	67.3	42.5	30.2	2.2
Strawberry	5.1	49.6	12.2	2.8
Pooled StDev	6.9	2.1	1.0	0.1
Water				
Bilberry	−11.3	184.3	0.0	0.9
Black currant	50.3	274.9	59.9	1.2
Elderberry	7.6	482.0	0.0	2.1
Lingonberry	12.4	152.8	0.0	1.1
Rose hip	−11.7	1760.2	824.9	6.8
Sea buckthorn	66.4	87.4	57.4	0.3
Strawberry	−20.7	118.9	0.0	0.6
Pooled StDev	7.5	9.9	0.9	0.1

4. Discussion

We used both a standard method to study antioxidant capacity, the ferric reducing ability of plasma (FRAP assay) [23], and an erythrocyte model, a modified CAP-e assay [22], to evaluate protective effects of berry extracts against oxidative stress.

The FRAP assay is an electron-transfer reaction based on the ability to reduce Fe^{3+} to Fe^{2+}. Because there is no oxygen radical in the assay it has been questioned how the results of the FRAP assay relate to *in vivo* antioxidant capacity. However, the FRAP assay is, in addition to for example the oxygen radical absorbance capacity (ORAC) assay, models frequently used as chemical methods for initial evaluation of antioxidant capacity and FRAP was therefore selected for this study.

In the erythrocyte assay, human erythrocytes are purified and incubated with berry extracts. Cells treated with berry extracts might be expected to gain some measure of protection against oxidative stress through the uptake of antioxidants [21]. In the assay, treated erythrocytes are washed to remove compounds loosely associated with

the cells, leaving only those that have achieved cell entry, where they might be either embedded or bound in the membrane lipid bilayer; this provides the opportunity to scavenge radicals effectively. Erythrocytes can compensate for intracellular oxidative stress and this accounts for some aspects of the cell uptake, distribution, metabolism of antioxidant compounds and the degree of protection they confer under more physiological conditions [21, 26, 27].

The results of the Folin-Ciocalteu assay (total phenols) and the FRAP assay (antioxidant capacity) are frequently correlated, as also reported in this study. The Folin-Ciocalteu assay has been criticised for not being a selective and reliable measure of total phenols due to its sensitivity for non-phenolic reducing compounds such as for example ascorbic acid and sugars [28, 29]. However, the contribution of ascorbic acid to total antioxidant activity can be determined, since one mole of ascorbic acid corresponds approximately to two moles of FRAP (in our assay we obtained and used the value 2.04). The contribution to the FRAP value of the ascorbic acid content of the rose hip water extract was thus calculated to be 825 μmol Fe^{2+}/L, which should be compared with the obtained total FRAP value which was 1760 μmol Fe^{2+}/L. Hence, there were significant amounts of other compounds that contributed to the antioxidant capacity of this extract (and also of other berry extracts containing ascorbic acid). The possibility of a significant contribution of ascorbic acid to oxidant protection in the erythrocyte model cannot be discarded, despite the absence of significant correlations between contents of ascorbic acid and protective effects of berry extract. All samples that contained some, albeit low levels of ascorbic acid, protected the erythrocytes agains oxidative stress. However, high levels of ascorbic acid, such as in the rose hip extracts, for example, actually caused a pro-oxidant effect. Such high levels are on the other hand not present in human blood.

Moreover, catechin was run as a positive control in both the FRAP and erythrocyte assay. The antioxidant capacity of catechin was 9182 ± 423 μmol Fe^{2+}/g dw at a concentration of 4.4 mg/mL and catechin at this concentration gave full protection in the erythrocyte assay. Using 1/8 of this concentration still resulted in a very high FRAP value (1148 μmol Fe^{2+}/g dw), which was higher than all of those obtained with the berry samples (except for the rose hip water extract), but protection was obtained at the same level as for sea buckthorn. Thus, the results of the measurements of the antioxidant capacity by the FRAP assay did not correspond to the degree of protection as determined in the erythrocyte assay.

Honzel et al. [21] and Blasa et al. [30] have also developed cellular antioxidant models based on erythrocytes, with the aim to predict the cellular protection and bioavailability of phytochemicals. In the assay used by Blasa et al. [30], the cellular antioxidant activity of pure flavonoids and vegetable extracts was determined and in the assay by Honzel et al. [21] the activity of complex natural products were tested. Both authors compared the erythrocyte data with the ORAC assay. The authors reported a lack of correlation between the different assays but suggested the use of both methods to provide a more complete evaluation of antioxidant activity.

In the studies by Honzel et al. [20] and Blasa et al. [30] the erythrocytes were washed with PBS (phosphate-buffered saline). We have previously performed a similar study where we investigated the protection against oxidative stress of different berry samples using a PBS based assay [31]. In that study we found superior protective effects of the rose hip extract. In the present study when SagM was used, the results were different: the rose hip extracts had a pro-oxidant effect. It is thus clear that the constituents of the incubations affect the results of the assay. The presence of glucose in the SagM solution is likely to maintain high GSH levels. The GSH maintenance system provides the reducing power to maintain cellular antioxidants in their reduced states. The uptake of glucose into the cells will ensure that cellular ATP and NADPHs levels will be optimized by glycolysis and the hexose monophosphate shunt (HMP) respectively [32]. This will in turn ensure sufficient cellular ATP and reducing power to maximise GSH levels through *de novo* synthesis and recycling via GSSG reductase. Crucially, GSH is also highly effective in the reactivation of ascorbic acid and the potentiation of its effects [32]. In addition, mannitol can function as an antioxidant, which also optimizes the preservation and stability of the erythrocytes [33, 34]. The use of SagM allows erythrocyte stability to be studied in an environment which is significantly closer to that of *in vivo* compared with the use of PBS. If the cells are maintained in an environment that either depletes GSH levels, or allows them to decay, their sensitivity to oxidative stress will be abnormally high. In such an environment, ascorbic acid can donate electrons to the plasma redox system and confer protection. High levels of ascorbic acid, such as in the rosehip extracts, may be beneficial in that case. Under conditions closer to that of *in vivo*, such as in the presence of SagM, ascorbic acid may still contribute as an electron donor at low levels, but not at the high levels found in the rose hip extracts. Interestingly, ascorbate is capable of pro-oxidant and antioxidant effects [35] and both ascorbic acid and mannitol may interact with iron. In a study by Gao et al. [13] the authors found that ascorbic acid acted as an antioxidant in peroxy radical-induced lipid

peroxidations, but as a pro-oxidant in the metal ion-induced lipid peroxidation. Hence, it is likely that the balance of the pro- and antioxidant effects of ascorbic acid is related to factors such as the general cellular reducing environment, ascorbate concentration and the presence of other oxidant and pro-oxidant agents [35].

It would be of considerable interest to study rose hip, bilberry, blackcurrant and sea buckthorn extracts in more detail and to evaluate the protective effect of extracts depleted of ascorbic acid. Sea buckthorn berries are, for example, known to contain high amounts of proanthocyanidins and isorhamnetin glycosides [6], which at digestion are hydrolysed to isorhamnetin, a phenolic compound that in previous studies has shown a high protective effect on erythrocytes [30]. We will in future work analyse the polyphenolic profile of the extracts and investigate the effects of single and complex mixtures of berry polyphenols on the protective effects on erythrocytes.

5. Conclusion

Analysis of ascorbic acid, total phenols and antioxidant capacity, as determined in the FRAP assay, cannot account for the protective effects of the different berry extracts that were obtained in the erythrocyte model. The lack of correlation between the chemical and biological assays is likely to be due to the complexity of the erythrocytes cellular model and the nature of the different compounds in the extracts tested. Whilst the clinical relevance of the erythrocyte assay requires further investigation, it remains the more biologically relevant of the assays employed and is likely to provide additional insights into the potential bioefficacy of different berry extracts, compared with conventional chemical assays.

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