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# Bioactive Berry Components: Potential Modulators of Health Benefits

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## ABSTRACT

This article focuses on biological activities that have been ascribed to berry antioxidant components other than vitamin C. We illustrate activities of berry polyphenol components, such as anthocyanins, flavonols and tannins, in model systems with potential relevance to cardiovascular health, cancers and glycaemic control. We present evidence that raspberry extracts but not blackcurrant extracts protected nitric oxide bioavailability in *ex vivo* rat carotid artery models. Nitric oxide is a key regulator of blood vessel dilation and can influence cardiovascular fitness. The raspberry extracts were more effective at lower concentrations than ascorbic acid and were as effective as quercetin metabolites known to be effective *in vivo*. Raspberry extracts also showed positive effects on platelet aggregation which could have beneficial effects on cardiovascular health. We present evidence that raspberry and cloudberry extracts can inhibit the proliferation of cancer cells grown *in vitro* and that raspberry extracts are more effective than cloudberry. As the cloudberry extracts are essentially similar in polyphenol composition to raspberry but much lower in anthocyanins and proportionally higher in ellagitannins, this raises questions about previous findings that suggested that ellagitannins were the major antiproliferative agents in raspberry. We screened berry extracts for their ability to inhibit pancreatic α-glucosidase activity, a key enzyme in starch digestion and blood glucose control. Raspberry, rowan, lingonberry and pomegranate juice extracts caused significant inhibition whilst strawberry, cloudberry, blackcurrant, blackberry and blueberry caused no inhibition at the concentrations used. A red wine extract significantly increased activity. A comparison of the polyphenol composition of the effective extracts suggests that the active agents may be more complex than previously thought.

**Keywords:** antioxidant, bioactivity, cardiovascular disease, cancers, diabetes, α-glucosidase, glycaemic control, polyphenols
**Abbreviations: AA**, ascorbic acid; **BC**, blackcurrant extract; **CARB**, carbachol; **GR**, digested raspberry sample; **NO**, nitric oxide; **PHE**, phenylphrine; **PPP**, platelet-poor plasma; **PRP**, platelet-rich plasma; **QG**, quercetin glucuronide; **QS**, quercetin sulphate; **RE**, raspberry extract; **SHRSP**, stroke prone spontaneously hypertensive rats

## INTRODUCTION

Over the last decades, a consensus has formed that the health benefits associated with a diet rich in fruit and vegetables may be derived, in part, from the intake of natural phytochemicals, some of which are potent antioxidants (Anon 2002). A theory has been proposed that antioxidant phytochemicals (mainly polyphenols, carotenoids and vitamin C), which are found in elevated levels in fruits especially berries (Machiex *et al*. 1990), augment natural antioxidant systems and protect against oxidative damage involved in the aetiology of cardiovascular disease, cancers and other diseases (Halliwell 1996). However, it is becoming clear that many classes of polyphenol compounds, such as anthocyanins, are poorly bioavailable and lack stability *in vivo* (Williamson and Manach 2005) and are therefore unlikely to provide antioxidant protection at the cellular level. Also, large proportions of ingested berry polyphenols remain in the gut and pass through to the large intestine where they may be fermented to support the indigenous microflora (Aura *et al*. 2005).

However, evidence continues to accrue that berries or polyphenol-rich berry preparations can influence the progression of neurodegenerative diseases, cancers, cardiovascular disease and diabetes *in vivo* (e.g. Ramassamy 2006; Erlund *et al*. 2008; Stoner *et al*. 2008; Tsuda 2008) even if an over-arching theory to explain their mechanism of action has not been formulated. This article illustrates biological activities of berry polyphenols that may be relevant to cardiovascular health, cancer and the modulation of glycaemic control.

## MATERIALS AND METHODS

### Plant material and extraction

Blackcurrants (*Ribes nigrum* L. breeding line 8982-6) were obtained from Bradenham Hall, Norfolk and blueberries (*Vaccinium myrtillus* L. cv. 'Berkeley') were grown at SCRI. Cloudberries (*Rubus chamaemorus* L.), lingonberries (*Vaccinium vitis-idaea*) and rowan berries (*Sorbus aucuparia* L. cv. 'Sahharnaja') were a gift from Dr. Harri Kokko, University of Kuopio, Finland and arranged via the European Union Northberry Project. Strawberries (*Fragaria ananassa* cv. 'Elsanta'), blackberries (*Rubus fructicosus* L.), pomegranates (*Punica granatum* L.) and red wine (Echo Falls, a Merlot variety wine from Mission Bell Winery, Madera, California, USA) were purchased from a local supermarket. Raspberries (*Rubus idaeus* L. cv. 'Glen Ample') were obtained from local farmers. Pure quercetin glucuronide (QG) and quercetin sulphate (QS) were gifts from Professor Alan Crozier, Division of Biochemistry and Molecular Biology, University of Glasgow. Polyphenol-rich extracts that lack sugars or ascorbic acid were extracted and used following solid phase extraction by the method described previously (McDougall *et al*. 2008a). Briefly, frozen fruit was homogenized in a Waring blender (6 × 20 s at full power) using an equal volume to weight of ice-cold 0.2% (v/v) formic acid in water. The extract was filtered through a glass sinter and applied to C18 solid phase extraction units (Strata C18-E, GIGA

Received: 4 February, 2009. Accepted: 28 April, 2009.

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units, Phenomenex Ltd., U.K.) pre-washed in 0.2% (v/v) formic acid in acetonitrile and then pre-equilibrated in 0.2% (v/v) formic acid in water. Unbound material, which contained the free sugars, organic acids, and vitamin C, was discarded. After extensive washes, the polyphenol-enriched bound extracts were eluted with acetonitrile. The C18-bound extracts were evaporated to dryness in a Speed-Vac (Thermo Fisher, Basingstoke, U.K.). A sample of raspberry extract was put through an *in vitro* digestion procedure (GR) to simulate human gastrointestinal digestion, recovered by solid phase extraction (see McDougall *et al*. 2005a for method) and used in certain studies.

## Anthocyanin and phenol assays

The total anthocyanin concentration was estimated by a pH differential absorbance method (Ribereau-Gayon and Stonestreet 1965). The absorbance value was related to anthocyanin content using the molar extinction coefficient calculated in-house for cyanidin-3-*O*-glucoside (purchased from ExtraSynthese Ltd., Genay, France). Phenol content was measured using a modified Folin-Ciocalteau method (Singleton and Rossi 1965) and, because all samples had passed through SPE treatment, we assume that they contain only phenolic components (George *et al*. 2005). Phenol contents were estimated from a standard curve of gallic acid.

## Platelet aggregation

Antecubital blood from healthy human volunteers (25 ml) was collected to investigate the effect of components on platelet aggregation. The blood was mixed with 3.15% (w/v) sodium citrate to obtain a ratio of 9:1 (blood: anti-coagulant) then centrifuged at 1000 rpm (MSE Mistral 2L centrifuge) for 18 min to obtain platelet rich plasma (PRP). The PRP was collected and the remainder was centrifuged at 2000 rpm for 10 min to obtain platelet poor plasma (PPP). The PPP was collected and the pellet containing the red blood cells discarded. Aliquots (450 µl) of PPP and PRP in a glass vial were placed in an aggregometer (Chrono-Log corporation Aggregometer, Labmedics Ltd, Manchester) to set PRP as giving no light transmittance and PPP as giving 100% light transmittance.

The extracts or vehicle (DMSO or Krebs) were added to 450 µl aliquots of PRP in glass vials. Raspberry extract (RE) was tested at final concentrations of 20 and 100 µg/ml, QG at 260 µg/ml (540 µmol/L), QS at 340 µg/ml (890 µmol/L) and ascorbic acid (AA) at 235 µg/ml (1334 µmol/L) and 100 µg/ml (556 µmol/L).

Adenosine diphosphate (ADP) was added at concentrations of 0.3, 0.6, 1.2, 2.5, 5.0, 10, 20 mmol/l to promote aggregation thus causing a reduction in turbidity The aggregometer prints a graphic recording of the turbidity change as a percentage of platelet aggregation. $EC_{50}$ values for aggregation in the presence and absence of extracts were calculated using the statistics package Prism. Paired t-tests were carried out to compare values in the absence and presence of extracts in blood from the same individual. n= 7 per group all results expressed mean ± standard error.

## Nitric oxide (NO) bioavailability

*In vitro* studies were carried out to measure nitric oxide (NO) bioavailability in vascular tissue according to previously published methods (Brosnan *et al*. 2002). Arteries were obtained from female stroke prone spontaneously hypertensive rats (SHRSP) which have relatively high levels of oxidative stress. The animals were obtained from colonies established in Glasgow. The carotid arteries were cleaned of connective tissue and cut into 2 mm rings. Then the rings were mounted in individual organ baths containing 10mls of Krebs solution for the measurement of isometric tension. The rings were suspended between two wire hooks attached to an isometric force transducer and were stretched to achieve approximately 1 g of resting tension (determined from previous work to produce optimal length-tension of this tissue). The baths were maintained at 37°C and bubbled with 95% oxygen and 5% carbon dioxide during the experiment.

Equilibration of 30 min was allowed before exposing the tissues to a test dose of 3 µmol/l phenylephrine (PHE) (an α1 adrenergic receptor agonist) and to 3 µmol/l carbachol (CARB) (a muscarinic agonist). After washing out thoroughly, the tissues were contracted with 10 mmol/l KCL twice with a 20 min wash out in between. After the second constriction with KCl, another wash out was carried out and approximately 30 min was allowed for the tissues to return to resting tone before further studies were undertaken.

A cumulative does-response curve to PHE (0.01 to 10 µmol/l) was then constructed which allowed the vessels to reach a stable plateau, and from this point relaxation to CARB (0.01 to 10 µmol/l) was recorded. The tissues were then washed out thoroughly before the test compounds were added. The extracts were added to give the following final concentrations, RE 90, 18, 1.8, 0.36 and 0.036 µg/ml, GR 1.8 µg/ml, QG 50 µg/ml (100 µmol/l) and 10 µg/ml (21 µmol/l) and QS 60 µg/ml (157 µmol/l), 12 µg/ml (31 µmol/l) and 3 µg/ml (8 µmol/l). AA was studied at 18 µg/ml (100 µmol/l). Additional control rings were incubated with vehicle alone (Krebs buffer for BC, RE, GR and AA and DMSO for QG and QS). The artery rings were incubated with the extracts for one hour then concentration response curves to PHE (0.01 to 10 µmol/l) and CARB (0.01 to 10 µmol/l) were repeated. After wash out the vessels were incubated with 100 µmol/l $N^G$-Nitro-L-Arginine methyl ester (L-NAME) a nitric oxide synthetase inhibitor. After 20 min incubation, another concentration response curve to PHE (0.01 to 10 µmol/l) was constructed.

The contractile responses to KCl at the start of the experiment allowed standardisation of the results obtained from the different rings in the different organ baths. The increase in tension caused by PHE in the presence of L-NAME compared to in the presence of the extracts provides a measure of basal NO bioavailability. NO bioavailability was calculated for each ring over the full PHE concentration-response curve and for each sample and was expressed as the area between the two curves (AUC g/g) using the calculation:

$$\left[\frac{\text{PHE + L - NAME}}{\text{KL}}\right] - \left[\frac{\text{PHE + extract}}{\text{KL}}\right] = \frac{\text{X}}{2} = \text{AUC g/g}$$

Unpaired *t*-tests using Minitab were carried out comparing NO bioavailability in the presence of extracts and the appropriate vehicle with Bonferroni correction tests for multiple comparisons. A value of $P < 0.05$ was regarded as significant. n = 6-10 per group results expressed mean ± S.E.M.

## Measurement of superoxide by xanthine/xanthine oxidase-lucigenin chemiluminescence

The effect of extracts on $O_2^-$ levels generated by xanthine/xanthine oxidase (XO) (White *et al.* 1996) was investigated by lucigenin chemiluminescence. AA was examined at final concentrations of 9 to 0.0009 µg/ml, RE at 9 to 0.0009 µg/ml, QG at 2 to 0.0002 µg/ml and QS at 3 to 0.0003 µg/ml. The extracts were added to 6 ml polyethylene (PE) vials containing 2 ml Krebs and XO (0.002 U), lucigenin (15 µmol/l) and xanthine (800 nM) were added to the PE vial and chemiluminescence read in a TRI-CARB 2100 TR liquid scintillation analyser (Packard BioSciences), set to count at 10-second intervals for three minutes beginning immediately after addition of XO. The results obtained in the presence of extracts were compared to standards in which no extract was present to determine if extract had reduced $O_2^-$ levels. A blank containing only 15 µmol/l lucigenin was subtracted from all readings. The data obtained was expressed as a percentage of $O_2^-$ inhibited in the presence of extract compared to the control, the calculation is:

$$A = \frac{\text{extract - blank}}{\text{control - blank}} \times 100$$

A = % $O_2^-$ detected in presence of extracts
100 – A = % inhibition of $O_2^-$ by extracts.

## Assessment of superoxide levels in SHRSP rat aorta

Lucigenin chemiluminescence was also used to investigate $O_2^-$ levels in the abdominal and thoracic arteries of SHRSP, in the presence and absence of extracts. The arteries were cleaned and cut in

to 4 mm rings and placed into a PE vial containing 2 ml Krebs. The weight of the tissue was recorded and the artery ring was incubated for one hour with extracts. AA was used at a final concentration of 130 µg/ml (738 µmol/l), RE at 90 µg/ml, QG at 240 µg/ml (500 µmol/l) and QS at 310 µg/ml (809 µmol/l). After an hour's incubation, $O_2^-$ was quantified against a standard curve generated from xanthine and XO and 15 µmol/l lucigenin.

## Cell proliferation and measurements of cell viability

Human cervical cancer (HeLa) cells were grown as a monolayer in Dulbecco's Modified Eagle Medium (DMEM) as described previously (Ross *et al*. 2007). Cells were harvested by trypsin treatment, washed with phosphate buffered saline (PBS) and resuspended in growth medium. They were then seeded to a final density of approximately 250,000 cells in 5 ml growth media inclusive of the different treatment solutions. Berry extracts were diluted in PBS then filter-sterilised and checked for phenol content to adjust for losses due to filtration. Cells were harvested over a 7 day period and cell number was assessed by counting and viability was assessed using a kit according the manufacturers instructions (Dojindo CCK-8 kit, from NBS Biologicals, Cambridge, UK). The antiproliferative effectiveness was estimated using an extrapolation of % inhibition values to yield the median effective dose ($EC_{50}$) at day 4 when the cells were actively growing and contained the largest proportion of viable cells.

## α-Glucosidase assay

The assay method has been described previously (McDougall *et al*. 2005b). Acetone powder from rat intestine (Sigma Chem Co. Ltd, product I1360) was used as a source of α-glucosidase. The release of *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside (Sigma Chem. Co. Ltd, product N1377) was measured after incubation at 37°C for 2 hours in the presence or absence of 50 µg/ml phenol content with suitable controls for each sample. Assays were carried out in triplicate.

## RESULTS AND DISCUSSION

Significant increases in nitric oxide (NO) bioavailability were seen from 90-0.36 µg/ml raspberry extract (RE) compared with the control buffer (**Fig. 1**). In contrast, blackcurrant extracts (BE) were not effective at 18 or 90 µg/ml. A raspberry extract that had been processed by an *in vitro* digestion procedure to simulate gastrointestinal digestion (GR) was similarly effective at 1.8 µg/ml. This suggests that the active ingredients of RE are not lost or that additional active compounds are formed during the digestion.

Ascorbic acid (AA) at 18 µg/ml (100 µmol/l) also increased NO bioavailability significantly compared to the control but to levels lower than 1.8 µg/ml or 0.36 µg/ml RE. Such comparisons of efficacy may be indicative but can only be valid if a full set of concentrations were performed for both components.

Both quercetin derivatives, QS and QG, significantly increased NO bioavailability compared to their vehicle DMSO (**Fig. 1**). However, the compounds showed different concentration effects. QG was more effective at the higher concentration (50 µg/ml than 10 µg/ml) but QS was equally as effective at 60 and 12 µg/ml, which suggests that QS is more potent. However, it should be noted that RE at 1.8 µg/ml and 0.36 µg/ml was considerably more effective than QS at 1.5 µg/ml.

The apparent effectiveness of the raspberry extract may be due to synergistic effects of a mixture of polyphenol components at lower levels as mixtures of QG and QS at concentrations well below those effective on their own caused significant increases in NO bioavailability (results not shown). Scavenging of superoxide has been postulated to protect NO bioavailability and maintain endothelial function by preventing NO conversion to peroxynitrile (Hamilton *et al*. 2001). Drugs such as Irbesartan that reduce superoxide levels, through blocking angiotensin II receptors, can influence hypertension (Brosnan *et al*. 2002).

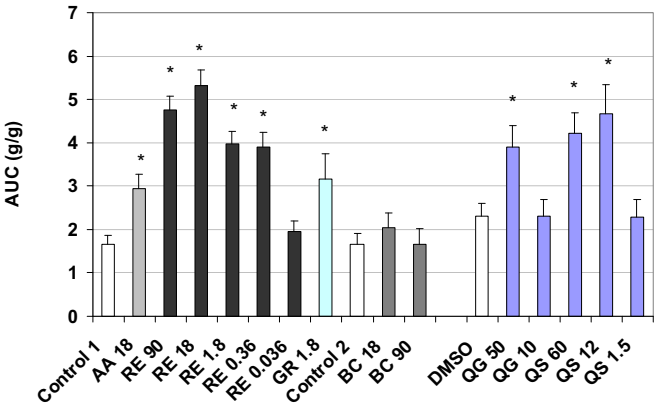
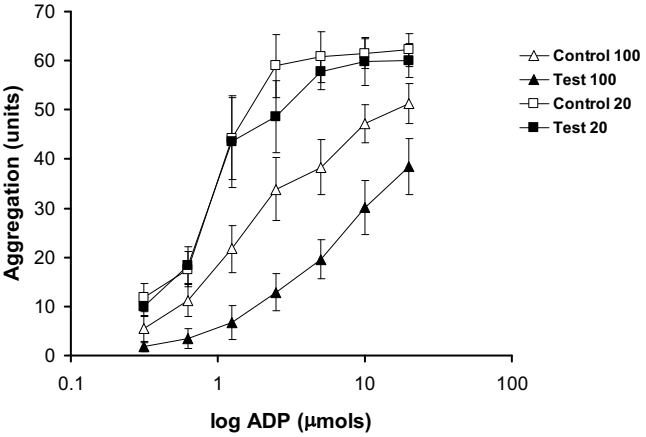



**Fig. 1 Effect of berry extracts on nitric oxide bioavailability in rat carotid artery.** * indicates a significant difference between the raspberry extracts and the control, using unpaired *t*-tests with Bonferroni correction tests for multiple comparisons using Minitab. *P*<0.05 was regarded as significant. AUC is the difference between the area under the curves for test and control samples as discussed in materials and methods.

**Table 1** Superoxide ($O_2^-$) scavenging activity of extracts

| Extract | $EC_{50}$ µg/ml | Number per group (n) | 95% Confidence Interval |
|---|---|---|---|
| Quercetin glucuronide | 0.012 | 4 | 0.006-0.022 |
| GR extract | 0.012 | 5 | 0.0055-0.024 |
| Raspberry extract | 0.026 | 6 | 0.012-0.059 |
| Quercetin sulphate | 0.047 | 5 | 0.020-0.103 |
| Ascorbic acid | 0.178 | 6 | 0.089-0.415 |

The raspberry extracts were effective scavengers of superoxide produced *in vitro* through xanthine/XO (**Table 1**). QG, RE and QS had the lowest $EC_{50}$ values but were not significantly different apart from being significantly more potent than ascorbic acid. The effective scavenging of superoxide by the extracts may partly explain their protection of NO bioavailability. However, in *ex vivo* studies with SHRSP rat aorta rings, all the extracts showed a slight tendency to reduce $O_2^-$ levels compared to controls but these differences were not statistically significant (paired *t*-tests at p>0.05). The noted difference between the potent *in vitro* superoxide scavenging and the lack of effect in the *ex vivo*




| Sample | n | µg/ml | $EC_{50}$ extract | $EC_{50}$ vehicle | P value |
|---|---|---|---|---|---|
| AA | 6 | 235 | 0.023 | 0.498 | 0.155 |
| RE | 7 | 100 | 1.617 | 7.512 | 0.001 |
| QS | 7 | 340 | 0.898 | 2.186 | 0.137 |
| QG | 7 | 260 | 1.047 | 0.657 | 0.701 |

**Fig. 2 The effect of raspberry extracts on platelet aggregation.** □ - Control RE 20 µg/ml, ■ - RE 20 µg/ml, Δ - Control RE 100 µg/ml, ▲- RE 100 µg/ml. For data in Table insert, paired *t*-tests were carried out to test for significance, *P*<0.05 was regarded as significant.

system may be due to the inability of the components to reach the intercellular sites of superoxide generation.

Raspberry extract caused a significant reduction in ADP-dependent platelet aggregation (**Fig. 2**) at 100 µg/ml but not at 20 µg/ml. Although ascorbic acid at 235 µg/ml caused an apparent reduction in platelet aggregation, only the raspberry extracts caused a statistically significant effect (Table, inset **Fig. 2**). Quercetin derivatives are known not to influence platelet aggregation at theses concentrations (Janssen *et al*. 1998) and the active components in the RE are unknown. In any case, the effective concentration of RE is unlikely to be encountered *in vivo*. Indeed, many studies suggest that anti-aggregation effects of flavonoids noted *in vitro* are due to concentrations that cannot be attained *in vivo* (Janssen *et al*. 1998).

Overall, it is apparent that raspberry extracts have considerable cardio-protective potential and were noticeably more effective than blackcurrant extracts *in vitro*. Blackcurrant extracts are particularly rich in anthocyanins but raspberry extracts contain anthocyanins and ellagitannins (Machiex *et al*. 1990; Kahkonen *et al*. 2001; Maatta-Riihinen *et al*. 2004). Indeed, ellagitannins have already been shown to be effective vasodilatory agents in rat aorta (Mullen *et al*. 2002) possibly due to their high relative antioxidant capacity (Ross *et al*. 2007 and references therein).

Given their poor absorption and stability (Williamson and Manach 2005), it is unlikely that ellagitannins or anthocyanins would be sufficiently bioavailable to influence cardiovascular events *in vivo*. On the other hand, QG and QS have been reported in human plasma and urine at around 7-10 µM (Grafe *et al*. 2001) with a reported half-life of 20-72 hours (Walle *et al.* 2001). This level approaches the concentrations found to be effective in this study and the synergistic effects of QG and QS on NO bioavailability may occur *in vivo* as both components are present in plasma flowing exposure to dietary quercetin derivatives (Walle *et al*. 2001).

Raspberry (**Fig. 3A**) and cloudberry extracts (**Fig. 3B**) inhibited the proliferation of HeLa cervical cancer cells in a dose-dependent manner. The raspberry extract gave an $EC_{50}$ of 21.0 µg/ml GAE whereas the cloudberry extract gave an $EC_{50}$ of 34 µg/ml GAE. Although the absolute value varied slightly with different experiments, the raspberry extracts were consistently more effective. Raspberry and cloudberry are closely related members of the *Rubus* family and have a similar polyphenol composition, differing mainly in the content of anthocyanins (Machiex *et al*. 1990; Kahkonen *et al*. 2001; Maatta-Riihinen *et al*. 2004). Previous work showed that an ellagitannin-rich raspberry sub-fraction was considerably more effective than the original extract or an anthocyanin-rich sub-fraction (Ross *et al*. 2007) which suggested that the ellagitannins were the predominant anti-proliferative agents in raspberry. However, if we consider that the cloudberry extract is effectively similar to an anthocyanin-depleted (and therefore relatively ellagitannin-enriched) raspberry extract, this suggests that the nature of the major anti-proliferative ingredients is much more complex. Indeed, in a separate study of the anti-proliferative effects of other *Rubus* species (McDougall *et al*. 2008a), we found that raspberry and arctic bramble extracts (which both contain anthocyanins and ellagitannins) were more effective than cloudberry extracts. Although cloudberry and raspberry contain similar ellagitannin profiles, they differ in the relative abundance of certain components (McDougall *et al*. 2008b) and it is intriguing to speculate that this may influence their biological activity or indeed their stability (Ross *et al*. 2007). Of course, cervical tissues are unlikely to come in contact with these components *in vivo* (Williamson *et al*. 2005) but colon cancer cells can be in direct contact with high levels of ingested polyphenols (e.g. Coates *et al*. 2007).

Berry extracts were screened to assess their ability to inhibit α-glucosidase activity *in vitro* (**Fig. 4**). Raspberry, lingonberry, rowan and pomegranate extracts showed significant inhibition whereas strawberry, cloudberry, blackcurrant and blueberry had no effect at the test concentration (50 µg/ml). Red wine extracts caused a significant increase in activity. Inhibition of α-glucosidase by drugs such as acar-

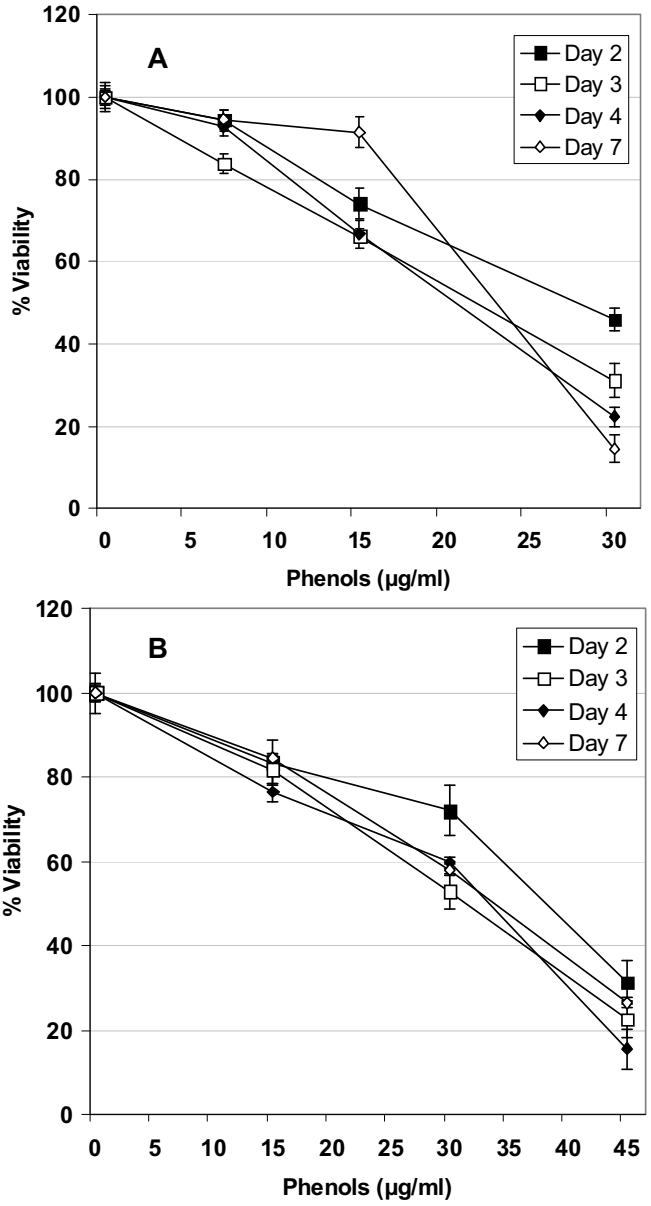



**Fig. 3 Effect of raspberry and cloudberry extracts on growth of HeLa cancer cells** (**A**) raspberry extracts; (**B**) = cloudberry extracts.

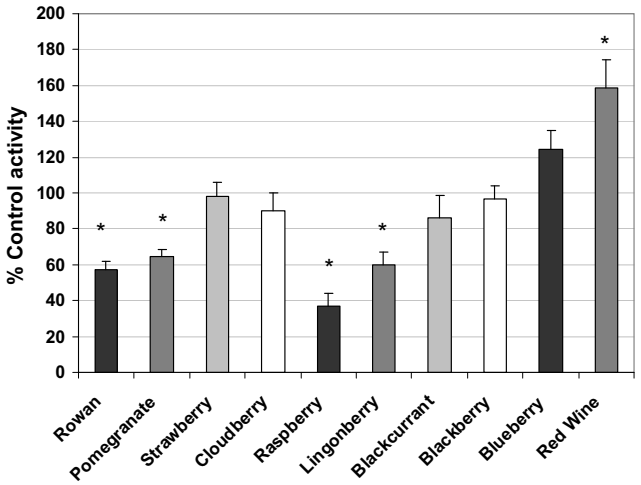



**Fig. 4 Effect of berry extracts on α-glucosidase activity *in vitro*.** * indicates a significant difference at $P<0.05$, paired *t*-tests.

bose is a therapeutically proven means of controlling post-prandial blood glucose levels in patients with poor glycaemic control due to type 2 diabetes (Toeller 1994). Polyphenolic extracts from plants have been reported as effective inhibitors of intestinal α-glucosidase activity with $K_i$ values similar to acarbose (Matsui *et al*. 2001). Inhibition of α-glucosidase has been reported for isolated anthocyanins (Matsui *et al*. 2002) and anthocyanin-rich extracts (McDougall *et al*. 2005b). Effective inhibition by anthocyanins acylated with hydroxycinnamic acids (Matsui *et al*. 2002) may be related to the inhibition of α-glucosidase by chlorogenic acid derivatives (e.g. Matsui *et al.* 2004). The wide distribution of chlorogenic acid derivatives may explain the ubiquitous discovery of varying extents of α-glucosidase inhibition in plant extracts and in certain berry extracts (Machiex *et al*. 1990).

However, elevated chlorogenic acid or anthocyanin content does not explain the pattern of α-glucosidase inhibition noted in this screen. Rowan and lingonberry extracts contain high levels of anthocyanins but so do blueberry and blackcurrant, which, like rowan, also have high levels of chlorogenic acids (Machiex *et al*. 1990; Kahkonen *et al*. 2001; Maatta-Riihinen *et al*. 2004; Hukkanen *et al*. 2006). Raspberry and the pomegranate extract contain lower levels of anthocyanins and substantial amounts of ellagitannins (Gil *et al*. 2000; Ross *et al*. 2007). However, ellagitannin-rich cloudberry, strawberry and blackberry extracts were ineffective. This initial screen suggests that α-glucosidase inhibition may be due to a combination of different components but further work is required to identify the active components. It is clear from model studies (McDougall *et al*. 2007) that considerable amounts of a wide range of berry polyphenols survive gastric digestion and could be available to modulate α-gluocosidase activity in the small intestine and influence blood glucose levels *in vivo*.

## CONCLUSIONS

Polyphenol-rich extracts from berries have bioactivities that influence model systems for cardiovascular disease, cancers and glycaemic control. Although antioxidant capacity may be involved in the mechanisms of sparing NO bioavailability and the anti-proliferative effects of berry extracts, it is not involved in the inhibition of α-glucosidase and other digestive enzymes.

## ACKNOWLEDGEMENTS

We thank Kilgraston School for allowing Victoria Lund to participate in work experience at SCRI. We thank Pat Dobson for technical help.

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