Mechanisms Underlying the Anti-Proliferative Effects of Berry Components in *In Vitro* Models of Colon Cancer

Emma M. Brown[a,*], Chris I.R. Gill[a], Gordon J. McDougall[b] and Derek Stewart[b]

[a]*Northern Ireland Centre for Food and Health, Centre for Molecular Biosciences, University of Ulster, Cromore Road, Coleraine, Northern Ireland, BT52 1SA, UK;* [b]*Plant Products and Food Quality Programme, Scottish Crop Research Institute, Mylnefield, Invergowrie, Dundee, DD2 5DA, Scotland, UK*

Abstract: Consumption of fruit and vegetables is associated with a decreased risk of several cancers, particularly colorectal cancer, possibly linked to their phytochemical content, which is of interest due to several proposed health benefits, including potential anticancer activity. Epidemiological data suggests that cancers of the digestive tract are most susceptible to dietary modification, possibly due to being in direct contact with bioactive food constituents and therefore investigating the effects of these bioactive compounds on the prevalent colorectal cancer is feasible. Berries are a common element of Western diets, with members of the *Rubus, Fragria, Sorbus, Ribes* and *Vaccinum* genus featuring in desserts, preserves, yoghurts and juices. These soft fruit are rich in bioactive phytochemicals including several classes of phenolic compounds such as flavonoids (anthocyanins, flavonols and flavanols) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids). Whilst there is little data linking berry consumption to reduced risk of colorectal cancer, *in vitro* evidence from models representing colorectal cancer suggests that berry polyphenols may modulate cellular processes essential for cancer cell survival, such as proliferation and apoptosis. The exact mechanisms and berry constituents responsible for these potential anticancer activities remain unknown, but use of *in vitro* models provides a means to elucidate these matters.

Keywords: Anti-proliferative, berries, colorectal cancer, phenolics.

INTRODUCTION

Epidemiological evidence suggests that diets rich in fruit and vegetables, including soft fruits such as berries which have a high content of phytochemical compounds, may contribute to a reduced risk of certain cancers, particularly colorectal cancer [1]. A diverse range of phytochemicals are found in berries, especially the phenolic compounds (including flavonoids, proanthocyanidins, stilbenes, hydroxycinnamic and hydroxybenzoic acids, lignans and hydrolyzable tannins) [2, 3], which are treated as xenobiotics in the body and therefore undergo extensive metabolism for rapid excretion [4].

Cultivated berries, such as blackberries, blueberries, strawberries, raspberries and cranberries, as well as more atypical "forest berries" including blackcurrant, bilberries, lingonberry and cloudberry [5], are commonly consumed as part of a Western diet in fresh or processed forms. A range of evidence supports the theory of anticancer properties of berries, although the mechanisms are still not fully understood but may include scavenging free radicals, induction of enzymes involved in metabolism of xenobiotics, regulation of gene expression, modulation of cellular signalling pathways including those involved in DNA damage repair, cell proliferation, apoptosis and invasion. This review aims to assess the *in vitro* evidence for anticancer activities and proposed mechanisms of action for whole berries, berry extracts and berry constituents, with an emphasis on phenolic compounds, in *in vitro* models of colon cancer. Whilst *in vivo* animal models are also important factors when investigating the anticancer effects of berries on colon cancer they have been extensively reported elsewhere [6-8] and will not be included in this review.

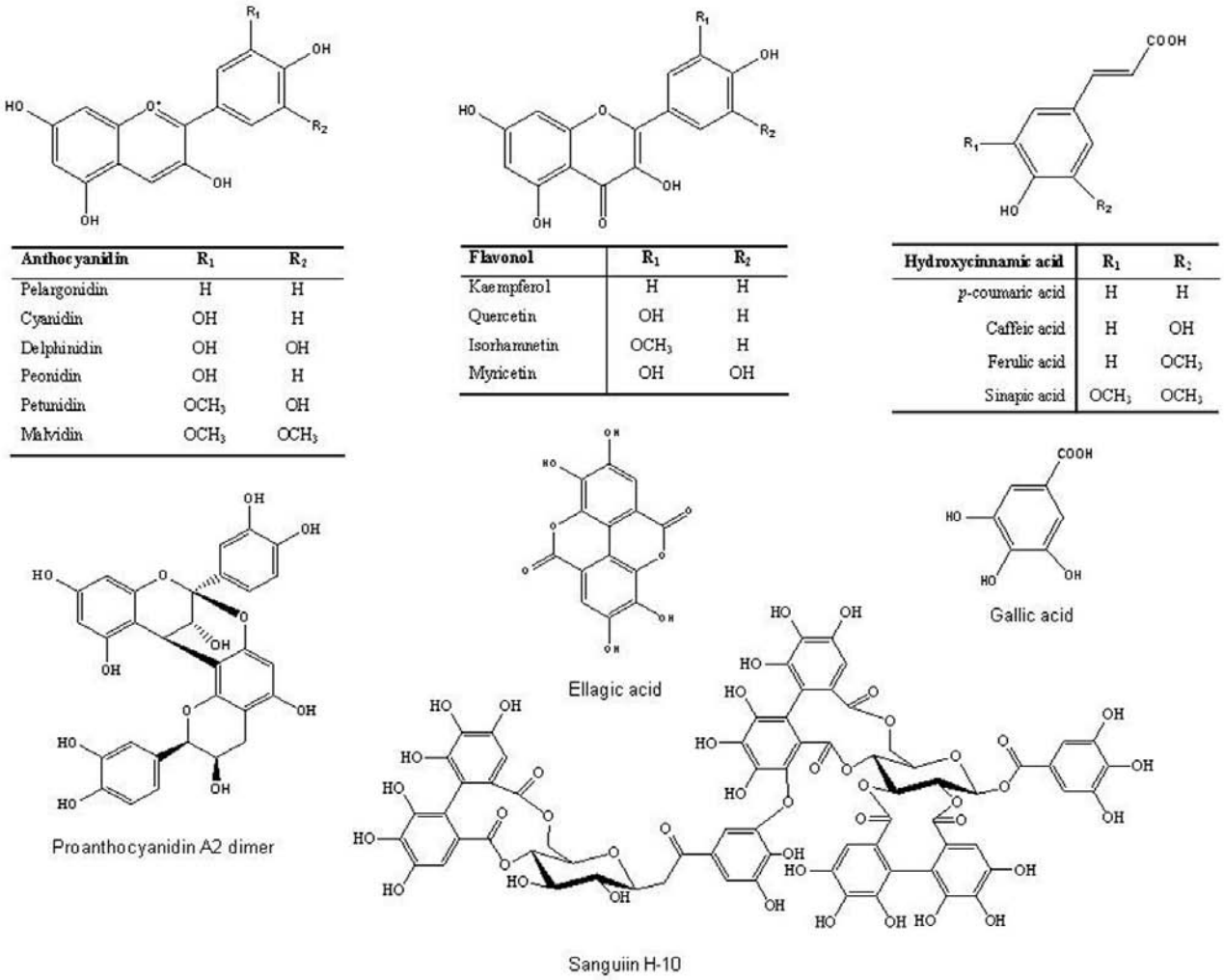
*Address correspondence to this author at the Northern Ireland Centre for Food and Health, Centre for Molecular Biosciences, University of Ulster, Cromore Road, Coleraine, Northern Ireland, BT52 1SA, UK; Tel: +44 28 703 23973; Fax: +44 28 703 23023; E-mail: em.brown@ulster.ac.uk

BERY BIOACTIVES AND COMPOSITION

Berries differ in the range and diversity of their major berry constituents, especially phenolic compounds. These are characterised by their number of aromatic rings and hydroxyl groups, and can be classified according to their structural diversity, ranging from simple single-aromatic ring compounds with low molecular weight to large complex molecules built up from multiple smaller structures [2-4]. In berries these compounds include phenolic acids (i.e. hydroxybenzoic and hydroxycinnamic acids) [9-11], stilbenes [12] and lignans [13]; flavonoids [4, 14] (including anthocyanins [15], flavonols [2, 4] and flavanols [4]) and condensed tannins (proanthocyanidins) [16-18] and hydrolyzable tannins (such as ellagitannins and gallotannins) [19-21]. Examples of some of these bioactives found in berries are provided in Fig. (**1a**, **1b**).

Phenolic content and composition in berries is influenced by genetic and environmental factors such as species and variety, cultivation methods, weather, time of and ripeness at harvesting, and conditions and time of storage [22-28]. The content and composition of phenolics varies with species, with berries of the same genus exhibiting characteristic profiles. In the *Vaccinum* genus, for example, the relative amounts of anthocyanins, flavonols, hydroxycinnamic acid (HCA) derivatives and proanthocyanidins differ between

1389-2010/12 $58.00+.00



Anthocyanidin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OH	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Flavonol	R_1	R_2
Kaempferol	H	H
Quercetin	OH	H
Isorhamnetin	OCH_3	H
Myricetin	OH	OH

Hydroxycinnamic acid	R_1	R_2
p-coumaric acid	H	H
Caffeic acid	H	OH
Ferulic acid	H	OCH_3
Sinapic acid	OCH_3	OCH_3

Fig. (1a, **1b).** Structures of some of the bioactive phytochemicals present in berries.

bilberry, cranberry and lingonberry [29, 30]. However in blueberries (*V. angustifolium* x *corymbosum* and *V. corymbosum*), and huckleberry (*V. parvifolium*), HCA derivatives predominate [31]. This can also be illustrated using the Rosaceae family of berries, which contains multiple genera including *Rubus*, *Fragaria* and *Sorbus*. Berries from the *Rubus* genus (cloudberry, raspberry and blackberry) primarily contain ellagitannins with differing levels of anthocyanins while strawberries (*Fragaria* genus) principally contain anthocyanins, closely followed by ellagitannins but also proanthocyanidins. However, fruits from the *Sorbus* genus, e.g. rowanberry, consist chiefly of HCA derivatives, with lesser amounts of flavonols and smaller amounts of anthocyanins [29]. In the Grossulariaceae family, fruits from the *Ribes* genus (including gooseberries, black and red currants) differ greatly in their anthocyanin content [29]. Other phenolics present in berries include flavanols such as monomers of (+)-catechin or (-)-epicatechin or as hydroxylated forms such as (+)-gallocatechin and (-)-epigallocatechin. Furthermore, stilbenes such as resveratrol are present in low quantities in berries including lingonberry, blueberry, cranberry and bilberry [32].

COLORECTAL CANCER

Colorectal cancer (CRC) is the third most prevalent cancer worldwide accounting for approximately 9.4% of global cancer cases with no apparent gender bias [1, 33]. Patterns of increased incidence of CRC have been observed in more developed parts of the world such as North America, Northern and Western Europe, Australia and New Zealand, and within the past 10 years CRC incidence in Japan has risen rapidly as this country has adopted a more Western lifestyle [34]. Conversely incidence is relatively low in less developed countries, including Africa and Asia, with an almost 25-fold variation in CRC incidence between these high- and low- incidence areas [35]. The differences in incidence rates between these less and more developed countries together with migrant information implicates environmental rather than genetic factors in the aetiology of this disease. Diet in particular may be a risk factor for CRC, with a lack of protective components in the Western diet hypothesised to be of greater risk than the presence of harmful components such as mutagenic heterocyclic amines. Thus dietary modification may be a feasible option to reduce CRC risk [36].

Development of CRC results from the accumulation of mutations or epigenetic changes that leads to transformation of normal colonic mucosa into colonic adenocarcinoma and subsequent carcinoma [37]. For sporadic tumours to develop, mutations in key genes of stem cells at the base of the crypt or early daughter cells must occur. This mutational event or "first-hit" is the initiating step in a pathway termed the *adenoma-carcinoma sequence* and involves genes such as *APC* (Adenomatous Polyposis Coli), K-*ras*, DCC (Deleted in Colorectal Cancer) and p53 Fig. (**2**) [38], and is characterised by three stages i) initiation involving exposure to or uptake of carcinogens resulting in permanent DNA damage, ii) promotion involving a lengthy process of abnormal cell replication forming a preneoplastic lesion and iii) progression of tumourigenesis involving gradual conversion of preneoplastic cells to malignant cells. During the tumour promotion stage, distinct genetic events resulting in deletion or silencing of genes occur, which result in hyper-proliferation of cells with the ability to evade apoptosis. As these cells continue to circumvent normal regulation, they are likely to develop subsequent mutations and progress from adenoma to carcinoma.

ANTICANCER ACTIVITY *IN VITRO*

Multiple *in vitro* studies have assessed the anticancer activities of berry bioactives, focussing on proliferation and apoptosis using whole berry extract, fractionated berry extract or purified/commercial berry components in a range of human colon cell lines representing different stages of colorectal cancer progression. These studies are summarised in Table **1**.

Dietary phenolics are able to modulate numerous cellular processes by up- or down-regulating key proteins involved in cell signalling pathways that control proliferation, differentiation and apoptosis, potentially resulting in anticarcinogenic activity [39, 40]. These potential anticancer effects appear to be stage specific, with cancer cell lines more sensitive to modulation than non-tumourigenic cells [41, 42]. However, determining the mechanisms behind the putative protective effects presents a challenge as the efficacy changes with dose, cell type used, exposure, the phenolic component selected, as an isolated compound [43] or in a mixture, all of which notably vary between studies (Table **1**).

Proliferation of cells occurs through a series of stages termed the cell cycle. During this process, DNA is replicated and separated into two daughter cells via mitosis. There are two phases of cell cycle: S phase (synthesis) where new DNA is synthesised and M phase (mitosis) where division occurs. These phases are separated by gap phases, G1 and G2. G1 precedes S phase, followed by G2 and then M phase. Throughout this cycle, a number of biological functions are occurring including DNA synthesis, proof-reading new DNA and correcting errors. Regulation of the cell cycle via checkpoints allows the cell to complete checks and make repairs to DNA etc before completing the cycle. However, in cancer cells, normal cellular growth and control is lost leading to abnormal cell cycle progression. Lack of cell cycle control leads to genetic instability (defective DNA repair) which increases transmission of genetic damage to subsequent generations of cells, and genomic instability characterised by chromosomal defects.

Decreased cell proliferation is commonly a result of cell cycle arrest. Control of the cell cycle is regulated by interactions between cyclins and cyclin-dependent kinases (CDKs), which, in turn, are controlled by CDK inhibitors (CDKIs), such as $p21^{WAF1}$ and $p27^{KIP1}$. Binding of cyclins to CDKs produces an active complex which promotes progression of the cell cycle through different stages. CDKIs can bind to this complex and deactivate it and thus halt the cell cycle. Cyclin A binds to CDK2 forming an active complex which allows progression of the cell through S phase of the cell cycle. Therefore, a decrease in the protein expression of cyclin A may account for an arrest in S phase after treatment with ellagic acid [43]. Further, increased expression of $p21^{WAF1}$ and $p27^{KIP1}$ genes along with decreased expression

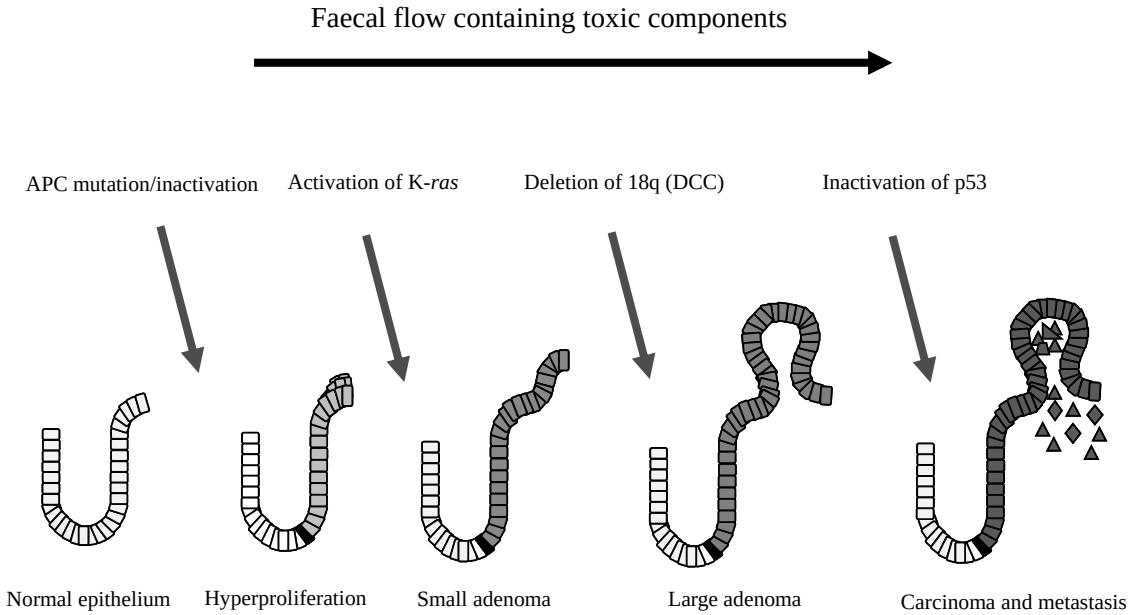


Fig. (2). Adenoma-carcinoma sequence - adapted from Fearon and Vogelstein [38].

Table 1. Evidence for the Anticancer Activity of Berries or Berry Components in *In Vitro* Models of Colorectal Cancer

Berry	Cell Line	Conc. and Exposure Time	End point	Observation	Protective/ Adverse Effect	Comment	Reference
Chokeberry anthocyanin rich extract (ARE)	HT29 NCM460 Non-tumorigenic (NT)	10 – 200 µg monomeric anthocyanin/mL 24 – 72 h	Proliferation	↓ Proliferation by 90% after 72 h and by 70% after 24 h at 50µg/mL Induced cell cycle block at G_0/G_1 and G_2/S phases of cell cycle at 50µg/mL corresponding with ↑ $p21^{WAF1}$ & $p27^{KIP1}$ &↓ cyclin A & B expression Growth of NT cells not affected (less than 10 % ↓ in proliferation)	+ *	Halt in cell cycle together with no decrease in cell viability after ARE indicative of cytostatic inhibition. Concentrations of ARE > 50 µg/mL ↓ cell viability.	[41]
Grape ARE Bilberry ARE Chokeberry ARE	HT29 + NCM460	25 – 75µg monomeric anthocyanin/mL 10 – 50 µg/mL MA 24 – 72 h	Proliferation	↓ Proliferation by 12, 35 & 55% after 24, 48 & 72 h at 75µg/mL ↓ Proliferation of NT cells by 46% after 72 h at 75µg/mL ↓ Proliferation by 15, 51 & 73 % after 24, 48 & 72 h at 75µg/mL ↓ Proliferation of NT cells after 72 h at all concentrations ↓ Proliferation by 52 & 70% after 48 and 72 h ↓ Proliferation of NT cells after 48 h at 50µg/mL and 72 h at all concentration	+ * + * + *	Inhibition of growth both dose and time dependent. Chokeberry ARE had a greater inhibition on proliferation based on monomeric anthocyanin content after 24 and 48 h incubations. Maximal ↓ in HT29 cells proliferation after 72 h with all ARE's but also observed ↓ in NT cell proliferation.	[42]
Cranberry Raspberry Strawberry Lowbush & highbush blueberry Blackcurrant Redcurrant Blackberry Cowberry Bilberry	HCT116	2 – 4 mg dry wt/mL 48 h	Proliferation & Apoptosis	All extracts ↓ proliferation after 48 h at 4mg/mL Bilberry was the most effective at ↓ proliferation No DNA fragmentation with any berry Anthocyanin-rich fraction of bilberry ↓ proliferation after 48 h at 400µg dry wt/mL Commercial delphinidin and cyanidin ↓ proliferation at 200 µM	+/- * +/- * +/- * +/- * +/- * +/- * +/- * +/- * +/- * +/- *	Cell viability was greatly reduced at the concentrations tested. ↓ cell proliferation probably due to necrosis rather than apoptosis. Anti-proliferative activity probably due to the anthocyanins delphinidin and cyanidin (present in bilberry)	[49]
Total cranberry extract (TCE) Total phenols Anthocyanins Proanthocyanidins (PA)	HT29 HCT116 SW480 SW620	200 µg/mL TCE 200 µg/mL 7.1 µg/mL 6.5 µg/mL 48 h	Proliferation	TCE ↓ proliferation by 78, 55 and 35% in HT29, HCT116 & SW620 cells. No ↓ proliferation in SW480. Total phenols ↓ proliferation by 92, 63, 61 & 60% in HCT116, SW620, HT29 & SW480 cells Anthocyanins and PA's ↓ proliferation to a lesser degree	+ * + * + * + *	Enhanced antiproliferative activity observed for total phenolics but devoid of sugars and phenolic acids. Synergistic effect between anthocyanins, PA's and flavonols.	[58]

(Table 1) contd….

Berry	Cell Line	Conc. and Exposure Time	End point	Observation	Protective/ Adverse Effect	Comment	Reference
Organically grown strawberries Conventionally grown strawberries	HT29	0.025 – 0.5 % dry wt 24 h	Proliferation	Organically grown strawberries ↓ proliferation by 60% at 0.5% DW Conventionally grown strawberries ↓ proliferation by 50% at 0.5% DW	+ * + *	↑ Vitamin C level associated with ↓ proliferation to a lesser extent than total extract; possible synergy between vitamin C and phenolics	[72]
Lingonberry Cloudberry Arctic bramble Strawberry (all devoid of free sugars, organic acids & vitamin C)	Caco-2	25 – 75 µg gallic acid equivalents/mL 72 h	Proliferation	↓ Proliferation by ~ 75% at 75µg/mL ↓ Proliferation by ~ 85% at 75µg/mL ↓ Proliferation by ~ 85% at 75µg/mL ↓ Proliferation by ~ 90% at 75µg/mL	+ * + * + * + *	High levels of ellagitannins may be responsible for inhibition of proliferation in cloudberry, arctic bramble and strawberry, whereas lingonberry inhibition was probably due to proanthocyanidins.	[57]
Rosehips Seabuckthorn Blueberry Blackcurrant Lingonberry Cherry Black chokeberry Raspberry	HT29	0.025 – 0.5 % dry wt 24 h	Proliferation	↓ Proliferation by ~ 70% at 0.5% DW ↓ Proliferation by ~ 70% at 0.5% DW ↓ Proliferation by ~ 65% at 0.5% DW ↓ Proliferation by ~ 65% at 0.5% DW ↓ Proliferation by ~ 70% at 0.5% DW ↓ Proliferation by ~ 50% at 0.5% DW ↓ Proliferation by ~ 40% at 0.5% DW ↓ Proliferation by ~ 50% at 0.5% DW	+ * + * + * + * + * + * + * + *	Inhibition of proliferation was dose dependant and degree of proliferation was different for different extracts. Possible synergy of phenolics and vitamin C.	[56]
Commercial berry anthocyanins and catechins	HCT116	6.25 – 100 µM 48 h	Proliferation	↓ Proliferation after 48 h exposure (-)-gallocatechin gallate > (-)-gallocatechin > (-)-epigallocatechin gallate > (-)-epigallocatechin > (-)-epicatechin gallate > (-)-catechin gallate at 100 µM Individual anthocyanins did not effect proliferation	+ *	Inhibition of proliferation was dose dependant Results suggest specific structure-activity relationships and the presence of galloyl groups Inhibition of COX enzymes with catechins, cyanidin and malvidin	[53]
Blackberry Black raspberry Blueberry Cranberry Red raspberry Strawberry	HT29 & HCT116	25 – 200 µg/mL 48 h	Proliferation & Apoptosis	↓ Proliferation black raspberry > blackberry > blueberry > strawberry > cranberry & raspberry at 200 µg/mL after 48 h incubation Black raspberry and strawberry induced apoptosis after 48 h at 200 µg/mL Slight increase in apoptosis for blackberry, raspberry & blueberry	+ * + * + NS	Dose dependant inhibition of proliferation which varied in potency between berry types Anthocyanins unique to black raspberry were cyanidin-3-sophoroside rhamnoside and cyanidin-3-sambubioside rhamnoside	[50]

(Table 1) contd….

Berry	Cell Line	Conc. and Exposure Time	End Point	Observation	Protective/ Adverse Effect	Comment	Reference
Tifblue Powderblue Brightblue Brightwell (anthocyanin-rich fractions)	HT29	50 – 150 µg/mL 6 h	Apoptosis	Tifblue & powderblue induced DNA fragmentation 50 – 150 µg/mL Brightblue and brightwell induced DNA fragmentation 50 – 100 µg/mL Caspase-3 activity was increased after treatment with all berry fractions	+ * + * + *	Brightblue & brightwell blue-berries induced cell death at 150 µg/mL probably by necrosis. Effect on prolif-eration was not investigated.	[48]
Bilberry Blackcurrant Cloudberry Lingonberry Red raspberry Strawberry Pure phenolics	HT29	5 – 60 mg/mL Prolif. 24 h Apop. 48 h 0–100 µg/mL	Prolifera-tion & Apoptosis	↓ Proliferation by 30 % at 10 mg/mL DNA fragmentation 20 – 60 mg/mL ↓ Proliferation by 20 % at 20 mg/mL ↓ Proliferation by 40 % at 40 mg/mL DNA fragmentation 40 – 60 mg/mL ↓ Proliferation by 30 % at 40 mg/mL ↓ Proliferation by 20 % at 40 mg/mL ↓ Proliferation by 30 % at 60 mg/mL ↓ Proliferation ≥ 75 µg/mL	+ * + * + * + * + * + * + * + * + *	Cell viability not affected indicating cytostatic inhibi-tion. Treatment with all berries induced $p21^{WAF1}$ mRNA expression but Bax expression was only induced after bilberry and cloudberry treat-ment. Bcl-2 ex-pressed in control cells only.	[44]
Ellagic acid	Caco-2 & CCD-112CoN normal colon cells	1 – 30 µM Prolif. Caco2 72 h CCD-112CoN 12 days Apop. 24, 48 & 72 h	Prolifera-tion & Apoptosis	↓ Proliferation maximally after 72 h by ~ 75 % ↓ Expression of Cyclin A and B1 ↑ Expression of Cyclin E Cell cycle arrest in S phase ↑ Cytoplasmic cytochrome *c* levels Induction of apoptosis annexin-V ↓ bcl-XL protein expression Activation of caspase 9 and 3 No effect on normal colon cells	+ * + * + * + * + * + * + * + * + *	Decreased prolif-eration and induc-tion of apoptosis via intrinsic path-way: down-regulation of 'pro-survival' protein bcl-XL & release of cytochrome *c* into cytosol linked with activation of caspase 9 & 3. Cyclin-mediated cell cycle arrest in S phase.	[43]

[a] Corresponds with reference number in main text and references section. * Significant result. + Protective effect (putative anticancer effect), - Adverse effect (putative harmful effect).

of cyclin A and B genes have been linked to a decrease in proliferation and halts in the cell cycle at G_0/G_1 and G_2/S phases after treatment with anthocyanin rich chokeberry extract [41]. The CDKIs $p21^{WAF1}$ and $p27^{KIP1}$ are both known inhibitors of complexes containing cyclins A and B, and therefore upregulation of their expression coupled with decreased expression of the cyclins is a probable explanation for decreased proliferation, especially when the extract did not effect cell viability. Conversely, non-tumorigenic cells under the same conditions did not show decreased cell proliferation or cell cycle perturbation of any kind demonstrating the specific action of the extract on tumour cells [41]. Similarly, a number of different berry extracts have shown differential inhibition of cell proliferation by inducing $p21^{WAF1}$ expression [44]. These studies demonstrate that similar results were achieved with different extracts at both physiologically relevant and non-relevant doses. *In vivo* such high concentrations of phenolics are unattainable through the diet and therefore results obtained at these doses are less relevant that those obtained at more plausible concentrations. For example, it has been suggested that 500 mg of phenolics consumed in the diet is diluted to approximately to 3 mM or 160 mg/L in the digestive bolus, and since it has been estimated that less than 1% of phenolics consumed are present

in the colon, less than 5 mg of phenolics would be present in the colon [45-47]. Therefore concentrations within the μg/mL dose range are physiologically feasible.

The growth of cancer cells is dependent on both unregulated proliferation and a lack of apoptosis. Induction of apoptosis, or programmed cell death, is considered an important target in cancer prevention as it is a function that is usually lost during cancer development. Two major apoptotic pathways have been identified. The extrinsic pathway depends on binding of ligands to cell surface "death" receptors, whilst the intrinsic pathway is induced by a variety of factors including DNA damage and involves mitochondrial function. Also termed the "mitochondrial pathway", a number of proteins feature in intrinsic apoptosis including Bcl-2 family proteins, which suppress apoptosis and are classified as "pro-survival", and other proteins such as Bax which is pro-apoptotic.

Apoptosis by the mitochondrial pathway also involves cytochrome *c* and caspases; specific proteases that degrade crucial cellular proteins during apoptosis. After release from the mitochondria, cytochrome *c* forms a complex with pro-caspase-9 resulting in caspase-9 activation. In turn, active caspase-9 activates pro-caspase-3, which is involved in degradation of nuclear DNA. Therefore, activation of caspase-9 and caspase-3 is indicative of apoptosis via the mitochondrial pathway. Indeed, pure ellagic acid and extracts of different varieties of blueberry have been shown to activate caspase-9 [43] and caspase-3 [43, 48] in Caco-2 and HT29 cells. However, when the concentration of two varieties of blueberry was increased, cell death was attributed to necrosis rather than apoptosis due to lack of DNA fragmentation [48]. Whilst multiple potential mechanisms were highlighted with purified ellagic acid, the maximal anti-proliferative effect was observed after a prolonged incubation time (up to 72 h) [43], whereas apoptosis was induced after just 6 h incubation time [48]. The average transit time in the colon is between 6 and 24 h, whereas the average lifespan of a colonocyte is approximately 6 days with more than half of this time spent migrating up the colonic crypt. Therefore, prolonged incubation times are less relevant to the *in vivo* situation where faecal contents are evacuated regularly and the cells are not in contact with dietary phenolics for this length of time. Thus these studies may be ambiguous with regard to magnitude of effect, which has been shown to increase with incubation time.

Induction of apoptotic pathways has been demonstrated by a range of berry extracts and purified berry compounds in a variety of colon cancer cell lines with different degrees of efficacy (Table **1**). For example, pure ellagic acid modulated the intrinsic pathway through reducing the expression of bcl-XL protein and activating caspases-3 and -9, corresponding with an increase in apoptotic cells and a decrease in proliferation [43]. Similarly both cloudberry and bilberry were able to induce apoptosis in HT29 cells, and interestingly the degree of DNA fragmentation (a late event in apoptosis) of cells exposed to both berry extracts was similar to the results for growth inhibition (i.e. both inhibition of proliferation and induction of apoptosis occurred at the same concentration range) [44]. Cloudberry and bilberry significantly induced pro-apoptosis Bax gene expression whereas pro-survival Bcl-2 gene expression was only detected in control cells suggesting decreased expression after berry treatment and indicating their involvement in the mitochondrial pathway [44]. Conversely, bilberry extract, which decreased proliferation, did not induce DNA fragmentation indicative of apoptosis in HCT116 cells [49] but greatly reduced cell viability, suggesting a necrotic action rather than apoptotic effect. These results, whilst interesting in terms of mechanistic information, were obtained with non-physiologically relevant doses and therefore the pertinence of the results may come into question.

A range of berry extracts have been shown to inhibit proliferation, but only a subset have induced apoptosis under the conditions tested [50]. Black raspberry and strawberry significantly induced apoptosis whereas blueberry, blackberry and raspberry showed only a slight increase in apoptosis, but this increase was not significant and could not fully explain the decrease in proliferation. Strawberry was the least effective in inhibiting proliferation but was a potent inducer of apoptosis, and therefore another mechanism must be responsible for inhibition of proliferation by the other berry types, but this was not investigated further [50].

Interestingly, berry extracts have been reported to induce apoptosis in cyclooxygenase 2 (COX-2) expressing HT29 cells [44], but not in HCT116 cells which lack COX-2 expression [49]. COX-2 is induced as a response to inflammatory stimulus and converts arachidonic acid into prostaglandins which are involved in the potentiation of inflammation [51]. Increased COX-2 activity can inhibit apoptosis via increased levels of the pro-survival protein Bcl-2 [52]. Pro-apoptotic berry extracts have been reported to reduce Bcl-2 levels in HT29 cells [44] and also induce DNA fragmentation, Bax expression, and internucleosomal degradation of DNA [50]. It is possible that the berry extracts are mediating their pro-apopotic effects through the COX-2 pathway resulting in a reduced expression of Bcl-2. For example studies have shown that berry components such as catechins and anthocyanins inhibit COX-2 activity *in vitro* [53], and ellagic acid treatment has resulted in reduced bcl-XL gene expression in Caco-2 cells which have low level Cox expression in their differentiated state [54]. The potential significance of COX-2 expression and apoptosis was illustrated by Tsujii and DuBois [52]. Rat intestinal epithelial (RIE) cells transfected with a COX-2 expression vector resulted in cells that became resistant to butyrate-induced apoptosis and expressed high levels of the pro-survival protein Bcl-2. By contrast, the parental RIE cells remained sensitive to butyrate-induced apoptosis and had no detectable levels of Bcl-2 protein [52]. Another study reported that HCA-7 cells (COX-2 expressing) showed growth inhibition in the presence of a COX inhibitor (SC-58125), while HCT116 cells (COX-2 non-expressing) were unaffected. Subsequent treatment of HCA-7 cells with prostaglandins reversed the growth inhibition, increased Bcl-2 protein expression resulting in a resistance to apoptosis [55]. If berry extracts are mediating their pro-apoptotic effects through the COX-2 pathway resulting in reduced expression of Bcl-2, then this may help explain why no effect is observed in the absence of COX expression. However it must be pointed out that these experiments generated the presence and absence of COX by utilising differ-

ent cell lines, rather than modulating expression of COX directly within the same cell line.

Whilst many studies have focussed mainly on anthocyanins, there is evidence to show that other berry constituents including flavonols, condensed and hydrolyzable tannins as well as phenolic acids exhibit antiproliferative activities *in vitro*. A common theme among these studies is the potential synergy between phenolic compounds, with themselves and other compounds present such as vitamin C. Caution must be used when comparing results however as different experimental conditions including incubation times and concentrations range from physiological to pharmacological, and differing results have been observed for different colon cancer cell lines. A general theme about the importance of dose is also raised in these models, with mechanisms such as apoptosis being induced at sub-toxic concentrations, but necrosis is observed at higher toxic doses.

DISCUSSION, KEY POINTS AND FUTURE CHALLENGES

This review summarises evidence from multiple sources that demonstrates that berry bioactives have potential anticancer effects in cellular models of colon cancer, but also highlights the fact that more information is required. *In vitro* models are a valuable tool for assessing the bioactivity of berry constituents and an abundance of evidence exists in terms of biological effect of phenolics on proliferation and apoptosis. The relevance of these results varies between studies and depends upon various factors such as incubation time, dose and extract used. The interpretation of results from these studies should be performed with caution; for example, a decrease in proliferation and an induction of apoptosis in a colon cancer cell line is an interesting result, however it may have been achieved using physiologically irrelevant concentrations. This review presents articles that provided a thorough description of scientific methodologies, including full description of controls and conditions used, to enable critical judgement on the use of different assays to measure various parameters, such as proliferation and apoptosis. Studies that included a more physiological dose range and incubation time were included, however some studies feature results obtained from extended incubation times (up to 72 h) and high doses (up to 60 mg/mL) and were used to compare differences in efficacy with dose and potential mechanisms involved. Ultimately this information provides starting points to focus more detailed mechanistic studies and enhances understanding of these potential anticarcinogenic compounds. However, information on structure: activity relationships is limited and what information is available has been gleaned from two different approaches. The first approach has compared extracts from a range of berry types in cellular models and then correlated activity against their phytochemical profiles to narrow down potential active components [49, 50, 56, 57]. This approach is obviously limited by the detail of knowledge on the phytochemical composition of the different extracts and can only indicate likely active components. The second approach often follows on from the first and attempts to confirm that fractions enriched in the "active" ingredients are effective. The results from this approach can be varied. In certain studies, the results seem clear cut. For example, when phenolic-rich extracts from lingonberry were found to be effective against colon cancer cells [57] subsequent fractionation suggested that the activity was predominantly present in a proanthocyanin-rich sub-fraction over the anthocyanin (and flavonol) rich sub-fraction. However, other fractionation studies have been less clear-cut. Seeram *et al.* [58] found that a phenolic-rich fraction from cranberry was more effective than juice preparations but that sub-fractions enriched in anthocyanins, proanthocyanins and flavonols were not as effective as the total extract. This suggests synergistic interactions between these components which influenced either their activity in the cellular model or perhaps enhanced the stability of the most active forms.

These studies seem to contradict previous studies that suggested that anthocyanin-rich extracts (AREs) were effective in various cellular models [41, 42]. However, these extracts contained at maximum 36 % anthocyanin content and the remaining phenolic content was not disclosed. It is interesting that the most effective ARE (from chokeberry) contained the largest proportion of non-anthocyanin phenols. However, further work with more purified AREs and pure individual anthocyanins confirmed that anthocyanins were effective inhibitors of colon cancer cell growth [59], but the authors also concluded that other phenolics could exert an additive interaction.

It is well known from animal studies [60] and human colostomy studies [61] that a large component of ingested berry phytochemicals survive digestion in the upper digestive tract and reach the colon in substantial doses. Therefore, the colonic epithelia can be in contact with doses of berry phenolic components which have been shown to have beneficial effects in cellular models. This is not the case for many other cancers as the systemic concentration of many phenolic compounds are usually very low due to low serum bioavailability [62]. However, it is also clear that phenolic components from berries are extensively metabolised to simpler phenolics by colonic microbiota during passage through the colon [63, 64]. Therefore, it seems reasonable to assume that the % recovery of original berry phenolics will drop as they pass further through the colon with a corresponding increase in microbiota-derived metabolites. Therefore, different parts of the proximal to distal colon will be exposed to different proportions of original phenolic components and their metabolites. In addition, inter-individual variation in the composition of the colonic microbiota will greatly influence the conversion and the spectrum of metabolites formed [65]. In this respect, it is also not clear if berry phenolics could have a beneficial effect on colon cancer incidence by supporting and modulating the diversity of the microbiota.

In light of this uncertainty, studies with isolated or mixtures of phenolics in their unmetabolised state, either as sugar conjugates or aglycones, may lead to ambiguous results in terms of magnitude of effect as the concentrations used (and the compounds used) may not be physiologically relevant to the colonic milieu *in vivo* [66]. Some studies have already established that colonic metabolites of phenolic compounds (similar to berry components) have bioactivities relevant to colon cancer [67, 68]. These studies are emerging and there is limited information about the magnitude of effect and mechanisms involved, but there is considerable

scope for comparison of such studies with pre-existing data. In addition, studies comparing the effectiveness of colonic metabolites with their parent compounds may have particular value. This was attempted by Bellion *et al*. [69] with phenolics from apple extract and fermented apple extract, which contained the parent compounds of quercetin glycosides and bacterial metabolites of quercetin, including 3,4-dihydroxyphenyl acetic acid respectively. This study demonstrated that the antioxidant potential of the fermented extract was diminished compared to that of the original extract by approximately 50% and subsequently the ability to protect against cellular reactive oxygen species and menadione-induced DNA damage was maintained but with less efficacy. Therefore, it is important that future studies in models of colon cancer examine the effects of physiological doses of relevant mixtures of phenolics and their colonic metabolites or breakdown products [70] and not focus on the parent compounds. Furthermore, it is clear that studies on cellular systems of apoptosis or proliferation are best accompanied by targeted studies to define the mechanisms involved and perhaps the uptake and metabolic fate of the active components within the cell.

In summary, the use of *in vitro* studies has helped elucidate some of the potential mechanisms involved in chemoprevention by berries and berry extracts in relation to cell proliferation. These mechanistic studies should continue in this post-genomic era alongside wider nutrigenomic studies (e.g. assessing genomic, metabolomic and proteomic changes in response to nutrients). The results from *in vitro* studies, although essential in research, cannot be extrapolated directly into *in vivo* systems, and therefore, further studies in both animal models and humans are essential to identify anticancer properties of berries. Interdisciplinary research is required to link basic and translational clinical research as briefly mentioned by Seeram [71] and such approaches will provide new insights into the bioactivity, bioavailability, metabolism, tissue specificity and accumulation of berry phenolics.

REFERENCES

[1] Stewart, B.W.; Kleihues, P., Eds. *World Cancer Report*; IARC Press: Lyon, **2003**.

[2] Seeram, N. P. Berries. In: *Nutritional Oncology*, 2nd ed.; Heber, D.; Blackburn, G.; Go, V.L.W.; Milner, J.; Eds.; Academic Press: London, U.K., **2006**; Chapter *37*, pp. 615-625.

[3] Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr*., **2004**, *79*(5), 727-747.

[4] Crozier, A.; Jaganath, I.B.; Clifford, M.N. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep*., **2009**, *26*(8), 1001-1043.

[5] Seeram, N.P. Berry fruits for cancer prevention: current status and future prospects. *J. Agric. Food Chem*., **2008**, *56*(3), 630-635.

[6] Stoner, G.D.; Wang, L.S.; Casto, B.C. Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries. *Carcinogenesis*, **2008**, *29*(9), 1665-1674.

[7] Stoner, G.D. Foodstuffs for preventing cancer: the preclinical and clinical development of berries. *Cancer Prev. Res. (Phila)*., **2009**, *2*(3), 187-194.

[8] Bishayee, A. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. *Cancer Prev. Res. (Phila)*., **2009**, *2*(5), 409-418.

[9] Mattila, P.; Hellström, J.; Törrönen, R. Phenolic acids in berries, fruits, and beverages. *J. Agric. Food Chem*., **2006**, *54*(19), 7193-7199.

[10] Liu, R.H. Potential synergy of phytochemicals in cancer prevention:mechanism of action. *J. Nutr*., **2004**, *134*(Suppl. 1), S3479-S3485.

[11] Clifford, M.N. Chlorogenic acids and other cinnamates: nature, occurrence and dietary burden. *J. Sci. Food Agric*., **1999**, *79*, 362-372.

[12] D'Archivio, M.; Filesi, C.; Di Benedetto, R.; Gargiulo, R.; Giovannini, C.; Masella, R. Polyphenols, dietary sources and bioavailability. *Ann. Ist Super Sanita*., **2007**, *43*(4), 348-361.

[13] Mazur, W.M.; Uehara, M.; Wähälä, K.; Adlercreutz, H. Phyto-oestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. *Br. J. Nutr*., **2000**, *83*(4), 381-387.

[14] Harborne, J.B.; Williams, C.A. Advances in flavonoid research since 1992. *Phytochemistry*, **2000**, *55*(6), 481-504.

[15] Clifford, M.N. Anthocyanins - nature, occurrence and dietary burden. *J. Sci. Food Agric.*, **2000**, *80*, 1063-1072.

[16] Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.*, **2000**, *80*(7), 1094-1117.

[17] Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Drilleau, J.F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety. *J. Agric. Food Chem*., **1998**, *46*, 1698-1705.

[18] Gu, L.; Kelm, M.A.; Hammerstone, J.F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R.L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr*., **2004**, *134*(3), 613-617.

[19] Clifford, M.N.; Scalbert, A. Ellagitannins, occurrence in food, bioavailability and cancer prevention. *J. Sci. Food Agric*., **2000**, *80*, 1118-1125.

[20] Foo, L. Y.; Porter, L. J. The structure of tannins of some edible fruita. *J. Sci. Food Agric.*, **1981**, *32*, 711-716.

[21] Määttä-Riihinen, K.R.; Kamal-Eldin, A.; Mattila, P.H.; González-Paramás, A.M.; Törrönen, A.R. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J. Agric. Food Chem*., **2004**, *52*(14), 4477-4486.

[22] Häkkinen, S.H.; Kärenlampi, S.O.; Mykkänen, H.M.; Törrönen, A.R. Influence of domestic processing and storage on flavonol contents in berries. *J. Agric. Food Chem*., **2000**, *48*(7), 2960-2965.

[23] Connor, A.M.; Luby, J.J.; Hancock, J.F.; Berkheimer, S.; Hanson E.J. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *J. Agric. Food Chem*., **2002**, *50*(4), 893-898.

[24] Skupień, K.; Oszmiański, J. Influence of titanium treatment on antioxidants content and antioxidant activity of strawberries. *Acta. Sci. Pol. Technol. Aliment.*, **2007**, *6*(4), 83-93.

[25] Häkkinen, S.H.; Kärenlampi, S.O.; Heinonen, I.M.; Mykkänen, H.M.; Törrönen, A.R. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J. Agric. Food Chem*., **1999**, *47*(6), 2274-2279.

[26] Deighton, N. Brennan, R.; Finn, C.; Davies, H.V. Antioxidant properties of domesticated and wild Rubus species. *J. Sci. Food Agric*., **2000**, *80*, 1307-1313.

[27] Castrejón, D.R.; Eichholz, I.; Rohn, S.; Kroh, L.W.; Huyskens-Keil, S. Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chem.*, **2008**, *109*, 564-572.

[28] Anttonen, M. J.; Karjalainen, R.O. Environmental and genetic variation of phenolic compounds in red raspberry. *J. Food Comp. Anal*., **2005**, *18*, 759-769.

[29] Kähkönen, MP.; Hopia, A.I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem*., **2001**, *49*(8), 4076-4082.

[30] Ek, S.; Kartimo, H.; Mattila, S.; Tolonen, A. Characterization of phenolic compounds from lingonberry (Vaccinium vitis-idaea L.). *J. Agric. Food Chem*., **2006**, *54*, 9834-9842.

[31] Taruscio, T.G.; Barney, D.L.; Exon, J. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest Vaccinium berries. *J. Agric. Food Chem*., **2004**, *52*(10), 3169-3176.

[32] Rimando, A.M.; Kalt, W.; Magee, J.B.; Dewey, J.; Ballington, J.R. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J. Agric. Food Chem*., **2004**, *52*(15), 4713-4719.

[33] Ferlay, J.; Bray, P.; Parkin, D.M., Eds. *GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide*; IARC Press: Lyon, **2004**.

[34] World Cancer Research Fund / American Institute for Cancer Research. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global* Perspective; Washington, DC, **2007**.

[35] Boyle, P.; Levin, B., Eds. *World Cancer Report*; IARC Press: Lyon, **2008**.

[36] Johnson, I.T. New approaches to the role of diet in the prevention of cancers of the alimentary tract. *Mutat. Res.*, **2004**, *551*, 9-28.

[37] van Breda, S.G.; de Kok, T.M.; van Delft, J.H. Mechanisms of colorectal and lung cancer prevention by vegetables: a genomic approach. *J. Nutr. Biochem*., **2008**, *19*(3), 139-157.

[38] Fearon, E.R.; Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell*, **1990**, *61*(5), 759-767.

[39] Ramos, S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J. Nutr. Biochem*., **2007**, *18*(7), 427-442.

[40] Surh, Y.J. Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer*, **2003**, *3*(10), 768-780.

[41] Malik, M.; Zhao, C.; Schoene, N.; Guisti, M.M.; Moyer, M.P.; Magnuson, B.A. Anthocyanin-rich extract from Aronia meloncarpa E induces a cell cycle block in colon cancer but not normal colonic cells. *Nutr. Cancer*, **2003**, *46*(2), 186-196.

[42] Zhao, C.; Giusti, M.M.; Malik, M.; Moyer, M.P.; Magnuson, B.A. Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. *J. Agric. Food Chem*., **2004**, *52*(20), 6122-6128.

[43] Larrosa, M.; Tomás-Barberán, FA.; Espín, J.C. The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *J. Nutr. Biochem*., **2006**, *17*(9), 611-625.

[44] Wu, Q.K.; Koponen, J.M.; Mykkänen, H.M.; Törrönen, A.R. Berry phenolic extracts modulate the expression of p21(WAF1) and Bax but not Bcl-2 in HT-29 colon cancer cells. *J. Agric. Food Chem*., **2007**, *55*(4), 1156-1163.

[45] Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr*., **2000**, *130*(8 Suppl. 1), S2073-S2085.

[46] Jenner, A.M.; Rafter, J.; Halliwell, B. Human fecal water content of phenolics: the extent of colonic exposure to aromatic compounds. *Free Radic. Biol. Med*., **2005**, *38*(6), 763-772.

[47] Bermúdez-Soto, M.J.; Larrosa, M.; Garcia-Cantalejo, J.M.; Espín, J.C.; Tomás-Barberan, F.A.; García-Conesa, M.T. Up-regulation of tumor suppressor carcinoembryonic antigen-related cell adhesion molecule 1 in human colon cancer Caco-2 cells following repetitive exposure to dietary levels of a polyphenol-rich chokeberry juice. *J. Nutr. Biochem*., **2007**, *18*(4), 259-271.

[48] Srivastava, A.; Akoh, C.C.; Fischer, J.; Krewer, G. Effect of anthocyanin fractions from selected cultivars of Georgia-grown blueberries on apoptosis and phase II enzymes. *J. Agric. Food Chem*., **2007**, *55*(8), 3180-3185.

[49] Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki,K.; Kobori, M. Induction of apoptosis in cancer cells by Bilberry (Vaccinium myrtillus) and the anthocyanins. *J. Agric. Food Chem*., **2003**, *51*(1), 68-75.

[50] Seeram, N.P.; Adams, L.S.; Zhang, Y.; Lee, R.; Sand, D.; Scheuller, H.S.; Heber, D. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. *J. Agric. Food Chem*., **2006**, *54*(25), 9329-9339.

[51] Watson, A.J. An overview of apoptosis and the prevention of colorectal cancer. *Crit. Rev. Oncol. Hematol*., **2006**, *57*(2), 107-121.

[52] Tsujii, M.; DuBois, R.N. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell*, **1995**, *83*(3), 493-501.

[53] Seeram, N.P.; Zhang, Y.; Nair, M.G. Inhibition of proliferation of human cancer cells and cyclooxygenase enzymes by anthocyanidins and catechins. *Nutr. Cancer*, **2003**, *46*(1), 101-106.

[54] Tsujii, M.; Kawano, S.; DuBois, R.N. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*(7), 3336-3340.

[55] Sheng, H.; Shao, J.; Morrow, J.D.; Beauchamp, R.D.; DuBois, R.N. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res*., **1998**, *58*(2), 362-366.

[56] Olsson, M.E.; Gustavsson, K.E.; Andersson, S.; Nilsson, A.; Duan, R.D. Inhibition of cancer cell proliferation *in vitro* by fruit and berry extracts and correlations with antioxidant levels. *J. Agric. Food Chem*., **2004**, *52*(24), 7264-7271.

[57] McDougall, G.J.; Ross, H.A.; Ikeji, M.; Stewart, D. Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown *in vitro*. *J. Agric. Food Chem*., **2008**, *56*(9), 3016-3023.

[58] Seeram, N.P.; Adams, L.S.; Hardy, M.L.; Heber, D. Total cranberry extract versus itsphytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *J. Agric. Food Chem*., **2004**, *52*(9):2512-2517.

[59] Jing, P.; Bomser, J.A.; Schwartz, S.J.; He, J.; Magnuson, B.A.; Gusti, M.M. Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. *J. Agric. Food Chem*., **2008**, *56*(20), 9391-9398.

[60] He, J.; Magnuson, B.A.; Giusti, M.M. Analysis of anthocyanins in rat intestinal contents - Impact of anthocyanin chemical structure on fecal excretion. *J. Agric. Food Chem*., **2005**, *53*(8), 2859-2866.

[61] Kahle, K.; Kraus, M.; Scheppach, W.; Ackermann, M.; Ridder, F.; Richling, E. Studies on apple and blueberry fruit constituents: do the polyphenols reach the colon after ingestion? *Mol. Nutr. Food Res*., **2006**, *50*, 418-423.

[62] Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.*, **2005**, *81*(Suppl. 1), S230-S242.

[63] Aura, A.M. Microbial metabolism of dietary phenolic compounds in the colon. *Phytochem. Rev*., **2008**, *7*, 407-429.

[64] Nurmi, T.; Mursu, J.; Heinonen, M.; Nurmi, A.; Hiltunen, R.; Voutilainen, S. Metabolism of berry anthocyanins to phenolic acids in humans. *J. Agric. Food Chem*., **2009**, *57*, 2274-2281.

[65] Gill, C.I.; McDougall, G.J.; Glidewell, S.; Stewart, D.; Shen, Q.; Tuohy, K.; Dobbin, A.; Boyd, A.; Brown, E.; Haldar, S.; Rowland, I.R. Profiling of Phenols in Human Fecal Water after Raspberry Supplementation. *J. Agric. Food Chem*., **2010**, article in press.

[66] Del Rio, D.; Costa, L.G.; Lean, M.E.J.; Crozier, A. Polyphenols and health: what compounds are involved? *Nutr. Metab. Cardiovasc. Dis*., **2010**, *20*, 1-6.

[67] Gao, K.; Xu, A.; Krul, C.; Venema, K.; Liu, Y.; Niu, Y.; Lu, J.; Bensoussan, L.; Seeram, N.P.; Heber, D.; Henning, S.M. Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity. *J. Nutr*., **2006**, *136*(1), 52-57.

[68] Larossa, M.; Luceri, C.; Vivoli, E.; Pagliuca, C.; Lodovici, M.; Moneti, G.; Dolara, P. Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. *Mol. Nutr. Food Res.*, **2009**, *53*, 1044-1054.

[69] Bellion, P.; Hofmann, T.; Pool-Zobel, B.L.; Will, F.; Dietrich, H.; Knaup, B.; Richling, E.; Baum, M.; Eisenbrand, G.; Janzowski, C. Antioxidant effectiveness of phenolic apple juice extracts and their gut fermentation products in the human colon carcinoma cell line caco-2. *J. Agric. Food Chem*., **2008**, *56*(15), 6310-6317.

[70] Coates, E.M.; Popa, G.; Gill, C.I.; McCann, M.J.; McDougall, G.J.; Stewart, D.; Rowland, I. Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer. *J. Carcinog,*. **2007**, *6*, 4-17.

[71] Seeram, N.P. Recent trends and advances in berry health benefits research. *J. Agric. Food Chem*., **2010**, *58*(7), 3869-3870.

[72] Olsson, M.E.; Andersson, C.S.; Oredsson, S.; Berglund, R.H.; Gustavsson, K.E. Antioxidant levels and inhibition of cancer cell proliferation *in vitro* by extracts from organically and conventionally cultivated strawberries. *J. Agric. Food Chem*., **2006**, *54*(4), 1248-1255.

Received: June 14, 2010 **Revised: October 11, 2010** **Accepted: October 15, 2010**