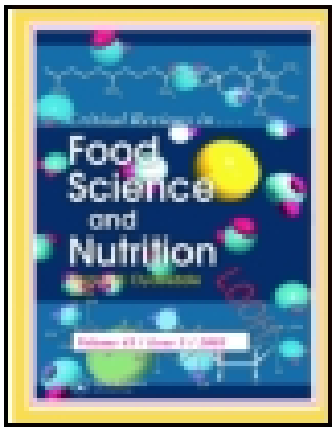

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Biological Activities of Plant Pigments Betalains

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Biological Activities of Plant Pigments Betalains.

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Abstract

Betalains are a family of natural pigments present in most plants of the order Caryophyllales. They provide colors ranging from yellow to violet to structures that in other plants are colored by anthocyanins. These include edible fruits and roots but also flowers, stems, and bracts. The recent characterization of different bioactivities in experiments with betalain-containing extracts and purified pigments has renewed the interest of the research community in these molecules used by the food industry as natural colorants. Studies with multiple cancer cell lines have demonstrated a high chemopreventive potential that finds *in vitro* support in a strong antiradical and antioxidant activity. Experiments *in vivo* with model animals and bioavailability studies reinforce the possible role played by betalains in the diet. This work provides a critical review of all the claimed biological activities of betalains, showing that the bioactivities described might be supported by the high antiradical capacity of their structural unit, betalamic acid. Although more investigations with purified compounds are needed, the current evidences suggest a strong health-promoting potential.

Keywords: Antiradical, Biological Activity, Cancer, Diet, Review

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2.1. Free radical scavenging and antioxidant activities.

2.2 Other activities *in vitro*.

3. Studies with cells.

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1. Introduction.

Betalains are water-soluble, nitrogen-containing pigments present in most plants of the order Caryophyllales. They are divided into two groups: the violet betacyanins and the yellow betaxanthins. Betacyanins present absorbance spectra centered at wavelengths around λ_m = 536 nm. Glycosylation and acylglycosilation of one or two hydroxyl groups are possible in betacyanins and complex pigment structures can be obtained (Heuer et al., 1994; Strack et al., 2003; Cai et al., 2005). In contrast, betaxanthins are yellow and no glycosylation has ever been reported. The absorbance spectra of betaxanthins are centered at wavelengths around λ_m = 480 nm. Both groups share betalamic acid as the structural and chromophoric unit. It is condensed with amines and aminoacids in betaxanthins and with *cyclo*-DOPA in the betacyanins (Gandía-Herrero et al., 2010a). Figure 1 shows the structures for betalamic acid, the betacyanins aglyca (betanidin) and the general structure for betaxanthins. The structure for the dopamine derived

betaxanthin (miraxanthin V) is shown and compared with other known bioactive metabolites like resveratrol and the anthocyanidin cyanidin.

Betalains are found in edible parts of the plants but also in the leaves (Wang et al., 2007), flowers (Gandía-Herrero et al., 2009a), stems (Schliemann et al., 1996) and bracts (Heuer et al., 1994). Anthocyanins and betalains are mutually exclusive and have never been found together in the same plant (Stafford, 1994; Brockington et al., 2011); in the Caryophyllales only the coloration of the *Caryophylaceae* and *Molluginaceae* is due to anthocyanins. Among the Caryophyllales plants, red beet roots (*Beta vulgaris*) (Hempel and Böhm, 1997), the fruits of cacti belonging to the genus *Opuntia* (mainly *Opuntia ficus indica*) (Felker et al, 2008; Osorio-Esquivel et al., 2011), the dragon fruits from *Hylocereus* cacti (mainly *Hylocereus polyrhizus*) (Wybraniec and Mizrahi, 2002 ; Wybraniec et al., 2007), and the Swiss chard (*Beta vulgaris*) (Kugler et al., 2004) are known edible sources of betacyanins and betaxanthins. Less common sources are the ulluco tubers (*Ullucus tuberosus*) (Svenson et al., 2008), the fruits of the *Eulychnia* cacti (Masson et al., 2011), and the berries from *Rivina humilis* (Khan et al., 2012). Certain *Amaranthus* species are also consumed cooked or fresh (Amin et al., 2006; Sang-Uk et al., 2009). Betalain-containing beet root extracts are used as the additive 73.40 in the 21 CFR section of the Food and Drug Administration (FDA) in the USA and under the E-162 code in the European Union to give a pink or violet color to foods and beverages (Martínez et al., 2006; Prudencio et al., 2008; Junqueira-Goncalves et al., 2011; Gandía-Herrero et al., 2012a). Figure 2 shows pictures of edible products containing betalains. New colorants derived from *Opuntia* fruits extracts (Mosshammer et al., 2006; Saénz et al., 2009; Obón et al., 2009) or containing individual pigments (Gandía-Herrero et al., 2010b) have also been proposed. The joint presence

of betaxanthins and betacyanins in the same parts of the plants generates orange to red shades depending on the pigment proportion (Schliemann et al., 2001; Kugler et al., 2004; Gandía-Herrero et al., 2005; Felker et al., 2008). Due to their hydrophilic nature, betalains are accumulated in the vacuoles of the cells that synthesize them, mainly in epidermal and sub-epidermal tissues of the plants (Wink, 1997). Interestingly, fungi of the genera *Amanita* and *Hygrocybe* (von Ardenne et al., 1974; Musso, 1979; Stintzing and Schliemann, 2007; Babos et al., 2011) produce betalain related pigments.

Betalains have in recent years shown promising bioactive potential. Early investigations revealed a strong free radical scavenging capacity of betalains purified from beet root (Escribano et al., 1998; Pedreño and Escribano, 2001). Subsequent research revealed the existence of an intrinsic activity present in all betalains that is modulated by structural factors (Cai et al., 2003; Gandía-Herrero et al., 2010a). Studies with different cell lines have demonstrated the potential of betalains in the chemoprevention of cancer (Wu et al., 2006; Sreekanth et al., 2007), and experiments *in vivo* have shown that very low concentrations of dietary pigments inhibit the formation of tumors in mice (Kapadia et al. 2003; Lechner et al., 2010). In humans, the plasma concentration of betalains after ingestion is sufficiently high to promote their incorporation into LDL and red blood cells, protecting them from oxidative damage and hemolysis (Tesoriere et al. 2003; 2005). However, most of the biological activities described have been reported according to studies with plant extracts with limited or none pigment purification. Although these studies are useful in identifing potential activities, isolated compounds are necessary to link the effects described with the structures responsible. In this work, the biological activities of betalains are

exhaustively reviewed, considering *in vitro* and *in vivo* experiments developed since the early description of its free radical scavenging activity more than a decade ago.

2. Activities *in vitro*.

2.1. Free radical scavenging and antioxidant activities.

Since the introduction of a feasible technology to determine the free radical scavenging potential of molecules and extracts by the Rice-Evans group (Re et al., 1999) the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical assay has become a standard technique in the evaluation of this activity. In betalains, the ABTS radical assay has gained relevance with respect to other similar methodologies such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay (Brand-Williams et al., 1995) and the ORAC (oxygen radical absorbance capacity) assay (Ou et al., 2001), or the direct reduction of Fe(III) to Fe(II) through the FRAP (ferric reducing antioxidant power) assay (Benzie and Strain, 1996). This is due to the use of a fully aqueous medium, the possibility of pH variation and the lack of signal interferences with fluorescent probes. Antioxidant and antiradical concepts are frequently not differentiated in the literature. It can be considered that antiradicals or radical scavengers are antioxidant molecules measured experimentally in the reduction of a radical. In this section the term antiradical will be used when the activity has been assessed through a radical-based assay (ABTS, DPPH, ORAC) independently of the original terms used by the authors. The antioxidant term will be restricted to experiments not involving stable radicals.

The first investigations that demonstrated a radical scavenging capacity in betalains were carried out separately with betacyanins and betaxanthins, extracted from *Beta vulgaris* (Escribano et al., 1998). Other works demonstrated activities in betalains purified from different sources: *Opuntia ficus-indica* (Butera et al., 2002), *B. vulgaris* roots grown under axenic conditions (Pavlov et al., 2002), and plants from the Amaranthaceae (Cai et al., 2003). In all cases the radical scavenging activity determined was higher than that detected for other well known compounds like ascorbic acid, catechin, and Trolox. Glucosylation of betacyanins was demonstrated to reduce the activity of the pigments (Cai et al., 2003) due to the blockage of one of the hydroxyl groups. However, the presence of these groups is not necessary to express activity, in contrast to the results obtained for flavonoids, where the absence of activity has been described in the dehydroxylated compounds *trans*-chalcone, flavone, flavanone, and isoflavone (Cai et al., 2006). Thus an "intrinsic activity" exists in all betalains studied which can be enhanced by the presence of hydroxyl groups, with an increase in terms of Trolox equivalence from 2.5 TEAC units to 4.0 for one and to 5.8 TEAC units for two hydroxyl groups (Gandía-Herrero et al., 2009b). The last value is higher than those found for other well known antioxidants like epigallocatechin gallate (EGCG) present in green tea (Rice-Evans et al., 1996; Stewart et al., 2005). Betalamic acid is the simplest structure with betalain properties and it also possesses antiradical and antioxidant activities, with a TEAC value of 2.7 (Gandía-Herrero et al., 2012b). Thus betalamic acid can be considered the bioactive unit of these pigments (Figure 1). Structure-activity relationships in betalains show that the antiradical activity for the simplest pigments is enhanced by the connection of the betalain characteristic electron resonance system with an aromatic ring, so increasing TEAC value by 0.4 units. If this is done to form indoline-

like substructures, like those present in betacyanins, the enhancement is higher, increasing the TEAC value by 1.6 units (Gandía-Herrero et al., 2010a).

Although the actual contribution of individual pigments is difficult to establish, the free radical scavenging activity of betalain containing extracts has been determined in several cases. The fruits of the cactus *Hylocereus polyrhizus* have revealed a strong free radical scavenging activity that was higher in peel extracts than in those obtained from the flesh, consistent with a higher content of betalains and flavonoids in the peel (Wu et al., 2006). Multiple clones of *Opuntia* plants showed that the TEAC values for cactus juices were close to those of red wine and green tea infusions (Stintzing et al., 2005). In addition to *O. ficus-indica*, recent attention has been focused on the fruits of other edible species like *O. joconostle* and *O. macrorhiza* (Osorio-Esquivel et al., 2011; Moussa-Ayoub et al., 2011; Morales et al., 2012). A complex profile of bioactive substances, including betalains has been described in these species, with extracts showing high antiradical and antioxidant activities. When purified betalain fractions were obtained, these exhibited higher activity than the flavonoid and phenolic containing fractions obtained from the same fruits (Osorio-Esquivel et al., 2011). Betalains from *Rivina humilis* were also partially purified and the activity of betacyanin and betaxanthin rich fractions were assayed (Khan et al., 2012). This confirmed both antiradical and antioxidant activities of the pigments and explained the antiradical effect detected in the fruit extracts. In the case of *B. vulgaris*, extracts from hairy root cultures have revealed a higher radical scavenging potential than intact plants. It has been proposed that this is due to an increased concentration of phenolic compounds, which may have a synergistic effect with betalains (Georgiev et al., 2010).

The redox properties of betalains have been studied by cyclic and differential pulse voltammetry. Reduction potentials were determined for purified indicaxanthin and betanin, and were higher in the case of the betacyanin (Butera et al., 2002). Strong antioxidant and antiradical betanin activities have been properly explained in terms of its electron donor capacity, calculating the bond dissociation energy and the ionization potential of the molecule (Gliszczyńska-Świgło et al., 2006). This explains the marked pH dependence found in free radical scavenging experiments. Increased pH values imply higher activity and higher TEAC values not only in the case of betanin. A comparative study with 15 natural and synthetic betalains showed a common trend in the pH dependence of the free radical scavenging activity (Gandía-Herrero et al., 2010a). This indicates the existence of a relevant deprotonation equilibrium in the expression of the activity and common to all betalains. The same tendency was found for free betalamic acid, and it has been linked to its nucleophilic capacity, determining a pK_a value of 6.8 (Gandía-Herrero et al., 2012b). A similar pH dependence of the free radical scavenging activity has been described for flavonoids. Deprotonation generates a phenolate anion in these molecules, which is a better electron donor and, thus, a more effective scavenger (Madsen et al., 2000; Muzolf et al., 2008).

Related to their spectroscopic properties and to their redox capacity to transfer electrons, betalains have been used as natural dyes in dye-sensitized solar cells (Zhang et al., 2008). These are one of the most promising devices for solar energy conversion due to their reduced production cost and low environmental impact (Narayan, 2012). The technology has been assayed with betalain containing extracts of *Beta vulgaris* roots (Zhang et al., 2008; Calogero et al., 2010), *Hylocereus* fruits (Ali and Nayan, 2010), *Opuntia* fruits (Calogero et al., 2010;

Calogero et al., 2012), and *Bouganvillea* bracts (Calogero et al., 2010; Hernandez-Martinez et al., 2011). While dye-sensitized solar cells containing anthocyanins and carotenoids as dyes have shown overall solar energy conversion efficiencies below 1%, betalain containing cells obtain conversion efficiencies of up to 1.7% under simulated sunlight conditions, which is comparable to that of natural photosynthesis (Calogero et al., 2009; Calogero et al., 2010; Zhou et al., 2011). On optimizing the performance of the solar cell and using purified betanin instead of raw extracts, the energy conversion efficiencies of the cells have recently been raised to 2.7% (Sandquist and McHale, 2011; Calogero et al., 2012).

2.2. Other activities *in vitro*.

In addition to their potent antioxidant and free radical scavenging activities, and probably in relation with them, the betacyanins betanin and betanidin isolated from *B. vulgaris* were able to inhibit the peroxidation of linoleic acid and the oxidation of LDL (low density lipoproteins). The effect was higher than that detected for other known antioxidants such as α-tocopherol and catechin (Kanner et al., 2001). These activities were assessed considering different oxidizers on linoleic acid emulsions and the oxidative susceptibility of human LDL obtained from healthy volunteers and microsomes obtained from turkey muscle tissue. Purified betalains have also been reported to be scavengers of hypochlorous acid, which is the most powerful oxidant produced by human neutrophils in inflammatory processes and to interfere with the activity of the myeloperoxidase enzyme responsible for its formation (Allegra et al., 2005).

Although the molecules responsible for the plant pigmentation seem to be erroneously identified (Kugler et al., 2004), aqueous extracts of Swiss chard (*B. vulgaris*) containing betalains were demonstrated to possess inhibitory activity on the enzyme acetylcholinesterase (Sacan and Yanardag, 2010). This enzyme is involved in the processes of neurotransmission by cleaving the neurotransmitter acetylcholine and its inhibition has been demonstrated to have therapeutic potential in the treatment of neurological disorders including Alzheimer's disease (Orhan, 2012).

3. Studies with cells.

Recent research with different cancer cell lines has demonstrated a high chemopreventive potential of betalain containing extracts. The use of purified pigments in some of the studies justifies the activities assessed and reinforces the biological potential of betalains. *Beta vulgaris* extracts have shown high chemopreventive effect in the induced Epstein-Barr early antigen activation assay *in vitro* using a cell line from lymphoma (Kapadia et al., 1996). Extracts significantly reduced cells viability, with the effect being ascribed to betalains. The activity was compared with other plant extracts, including an anthocyanin-rich cranberry extract, exhibiting the maximum activity. A limited cytotoxicity of *B. vulgaris* extracts has also been demonstrated for human prostate and breast cancer cell lines (Kapadia et al., 2011).

Extracts obtained from *Opuntia ficus-indica* proved to be an effective growth inhibitor and apoptosis inductor in several cell lines of immortalized ovarian and cervical epithelial cells and ovarian, cervical, and bladder cancer cells (Zou et al., 2005). The effect of the cactus pear

solution was dose and time-dependent. Cactus extracts were able to inhibit the cancer cells growth and affect their morphology with concentrations of 5% of the fruit extract in the cell culture. A higher concentration was necessary (10%-25%) for the apoptotic effect. However, the use of raw extracts avoided the identification of active compounds. Betalains from the berries of *Rivina humilis* were tested regarding their effect on hepatocellular carcinoma cells (Khan et al., 2012). In this case, the betalains were partially purified and betaxanthins and betacyanins separately showed dose dependent cytotoxicity after 24 and 48 hours respectively.

Extracts from the fruit of the cactus *Hylocereus polyrhizus* revealed an inhibitory activity of the growth of melanoma cancer cells (Wu et al., 2006). The inhibition was dose-dependent and higher in peel than in flesh extracts, in relation with a higher content of flavonoids and betalains. Remarkably, in the same experiment pure betanin was assayed, revealing a strong inhibition of the proliferation of the melanoma cancer cells. Pure betanin was also used against a human chronic myeloid leukemia cell line in a different study (Sreekanth et al., 2007). The addition of betanin implied the inhibition of the cell growth in a dose dependent manner with an IC_{50} of 40 μM after 24 h of incubation. Betanin enters the cells and alters the mitochondrial membrane integrity. This ultimately leads to the activation of caspases and to nuclear disintegration. The biochemical alterations were reflected in morphological changes of the cells which were followed by scanning and transmission electron microscopy and flow cytometry. Cells entered apoptosis, in a clear *in vitro* demonstration of the anti-cancer potential of betalains. Table 1 summarizes all the studies developed with betalains which involved cancer cells, identifying the corresponding cell lines.

In addition to the effect on cancer cell lines, *B. vulgaris* extracts have been found to dose-dependently suppress the degradation of tryptophan and the production of neopterin in human peripheral blood mononuclear cells involved in the inflammatory response (Winkler et al., 2005). Cells were obtained from healthy donors and stimulated in the presence of the betalain-containing extracts. The cell response was reduced by the presence of the extracts, suggesting that the *B. vulgaris* juice possesses compounds with immunosuppressive and anti-inflammatory activities. Human red blood cells also showed increased resistance to hemolysis when they were incubated with growing concentrations of purified indicaxanthin and betanin *in vitro* (Tesoriere et al., 2005). Furthermore, low density lipoproteins (LDLs) isolated from healthy humans have been demonstrated to incorporate purified indicaxanthin and betanin after *ex vivo* incubation (Tesoriere et al., 2003). LDLs enriched with the pigments were more resistant than native LDLs to copper-induced oxidation, with indicaxanthin being more effective than betanin in the protection from the oxidative damage.

4. Studies with animals.

In vivo anti-tumor formation activity in mouse skin has been demonstrated for *Beta vulgaris* extracts. The extracts were orally administered to the animals in drinking water after topical tumor induction (Kapadia et al., 1996). Results showed a significant decrease in the incidence and number of papillomas found in the mice skin. In the same study, lung tumor formation was induced to mice, and inhibited by the oral administration of *B. vulgaris* extracts. A 60% reduction in the number of mice with adenomas was observed, with those showing the

tumor seeing its number reduced by 30%. Skin tumor formation chemically induced and promoted by UV-B light was also inhibited after the oral administration of *B. vulgaris* extracts in mice (Kapadia et al., 2003). In addition, animals that followed the treatment also showed a reduced splenomegaly. In the case of induced tumors in the liver, beet extract oral administration reduced the tumor incidence to 40%, showing a potent cancer chemopreventive activity in the model animals.

Analogously, chemoprevention against induced esophageal carcinogenesis was also demonstrated in rats for *B. vulgaris* extracts administered orally (Lechner et al., 2010). Results showed a reduction of 45% in the number of papillomas, limiting cell proliferation, angiogenesis, and inflammation. It was hypothesized that betalains antioxidant activity reduced the level of reactive oxygen species to levels that were too low to stimulate the anomalous proliferation.

In addition, dose-dependent protection of the adverse effects of γ-ray irradiation in rats has been demonstrated *in vivo* for *Beta vulgaris* extracts, and the effect has been ascribed to betalains (Lu et al., 2009). Orally administered extracts partially restored the normal biochemical levels of the altered parameters caused by the irradiation, including catalase, superoxide dismutase and lipid oxidation activities in liver, spleen and kidney. Furthermore, the prevention of the decline in the spleen and thymus index in irradiated mice led the authors to suggest that betalains could partially restore the immunological function and improve the pathological status *in vivo*.

Although the effect of betalains cannot be identified, aqueous extracts of *Opuntia ficus-indica* were able to inhibit tumor growth in a nude mouse of ovarian cancer model compared with untreated animals (Zou et al., 2005). The extract was administered by injection and

compared with the chemopreventive agent N-(4-hydroxyphernyl) retinamide (4-HPR), used in ovarian cancer clinical trials. Both cactus pear extracts and the chemopreventive agent reduced the tumor size in a comparable manner. *Opuntia* fruits extracts have also demonstrated their potential in the protection and recovery of the liver after damage has been induced. Hepatotoxicity by carbon tetrachloride (CCl_4) in rats was limited in animals fed with the betalain containing extracts both after and before the damaging treatment (Galati et al., 2005). The oral administration of the extract promoted liver recovery at histochemical and biochemical levels. The same effect was described for betalain-containing extracts of whole plants of *Amaranthus spinosus*, and it has been proposed that the mechanism of hepatoprotection was due to its antioxidant activity (Zeashan et al., 2008; 2009).

Interestingly, other activities have also been described for betalains. Purified pigments extracted from *Portulaca oleracea* have demonstrated their capacity to reverse induced learning and memory impairments produced by D-galactose in mice (Wang and Yang, 2010). In comparison with ascorbic acid, orally administered betalains showed a more pronounced effect in ameliorating cognition deficits in mice and restored the normal biochemical levels of the relevant enzymes, being proposed a neuroprotective effect. *Amaranthus spinosus* and *Boerhaavia erecta* extracts containing betalains have demonstrated antimalarial activity in an *in vivo* model assay in mice (Hilou et al., 2006). The aqueous extracts were able to inhibit the growth of inoculated parasites in a dose dependent manner. These plants are used in the traditional medicine against *Plasmodium falciparum* infections in humans (malaria). The authors pointed to the betacyanins betanin and amaranthin as possible molecules responsible for the assessed activity at the same time that they acknowledged the need to perform experiments with

purified compounds. *Opuntia ficus-indica* extracts and its main pigment, indicaxanthin, in a pure form were demonstrated to reduce the contractility of the ileal longitudinal muscle obtained from mice (Baldassano et al., 2010; 2011). The authors propose the usefulness of the finding in the regulation of the intestinal motility in related disorders and describe the mechanism of action. It implies the inhibition of phosphodiesterases enzymes and the increase of the levels of cAMP, which leads to a decrease in intracellular Ca^{2+} concentration, which ultimately promotes the smooth muscle relaxation.

Bioavailability studies of betalains after oral administration in humans indicate that the model betalains, betanin and indicaxanthin remain in the body and are able to play a health-promoting function, so improving the body redox status (Tesoriere et al., 2004; Frank et al., 2005). Maximum plasma concentrations are reached 3 hours after consumption, with a decline corresponding to first-order kinetics. After this time, betalains can be incorporated into the red cells *in vivo* (Tesoriere et al., 2005). 12 h after ingestion they are completely eliminated, with a urinary excretion of 76% in the case of the betaxanthin, but highly limited in the case of betanin. This indicates metabolization of the pigment and its transformation to other compounds, including betalamic acid, as demonstrated in simulated digestion studies (Pavlov et al., 2005; Tesoriere et al., 2008). However, the inability of part of the population to metabolize betanin has also been described, excreting it to a high extent in the urine. This is known as beeturia and although its mechanism is not well understood, its incidence is higher in iron-deficient subjects (Watson et al., 1963; Sotos, 1999; Mitchell, 2001).

5. Conclusions.

The description of the betalains free radical scavenging activity implied the renaissance of the interest on these molecules by the research community. Since then, multiple articles with claims regarding the biological activity of betalains have been published, including studies on the chemoprevetion of tumor formation. Considering their demonstrated safety (Schwartz et al., 1983; Khan et al., 2011), the recent bibliography indicates the potential of betalains for the food, pharmaceutical and cosmetic industries.

Promising results have been reported for tumor prevention *in vivo* and the possible role played by betalains in the diet. However, the use of extracts limits the conclusions drawn, the hypothesis on the mechanisms involved and the therapeutic potential of the assays. Currently, studies with purified pigments are scarce but they provide exciting conclusions. Increasing of these studies would help to establish the actual role played by betalains alone or in cooperation with other compounds. Much caution must be taken regarding the possible application of the biological activities described in natural molecules, but the results for betalains are promising in terms of their health-promoting potential.

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Table 1. Studies with betalain-containing extracts involving cancer cells. The cell line identification, the source of betalains, the use of purified pigments and the corresponding reference are shown in each case.

Cell line	Betalains source	Effect	Pigment purification	Reference
Raji (lymphoma, human)	*Beta vulgaris*	Reduced cell viability	No	Kapadia et al., 1996
IOSE (ovarian epithelium, human)	*Opuntia ficus-indica*	Growth inhibition	No	Zou et al., 2005
OVCA420 (ovarian cancer, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	No	Zou et al., 2005
SKOV3 (ovarian cancer, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	No	Zou et al., 2005
TCL-1 (cervical epithelium, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	No	Zou et al., 2005
HeLa (cervical cancer, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	No	Zou et al., 2005
Me180 (cervical cancer, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	No	Zou et al., 2005
UM-UC-6 (bladder cancer, human)	*Opuntia ficus-indica*	Growth inhibition	No	Zou et al., 2005
T24 (bladder cancer, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	No	Zou et al., 2005
B16F10 (melanoma, mouse)	*Hylocereus polyrhizus*	Growth inhibition	No	Wu et al., 2006
B16F10 (melanoma, mouse)	Unspecified (commercial)	Growth inhibition	Purified betanin	Wu et al., 2006
K562 (leukemia, human)	*Opuntia ficus-indica*	Growth inhibition, apoptosis induction	Purified betanin	Sreekanth et al., 2007
PC-3 (prostate cancer, human)	*Beta vulgaris*	Growth inhibition	No	Kapadia et al., 2011
MCF-7 (breast cancer, human)	*Beta vulgaris*	Growth inhibition	No	Kapadia et al., 2011
HepG2 (liver cancer, human)	*Rivina humilis*	Reduced cell viability	Partial	Khan et al., 2012

Figure Captions

Figure 1. Structures for betalamic acid, the betacyanins aglyca (betanidin), and the general structure for betaxanthins. R^1 and R^2 are lateral residues present in amines or amino acids. For comparative purposes, the structure for the diphenolic betaxanthin Miraxanthin V is also shown together with resveratrol and cyanidin.

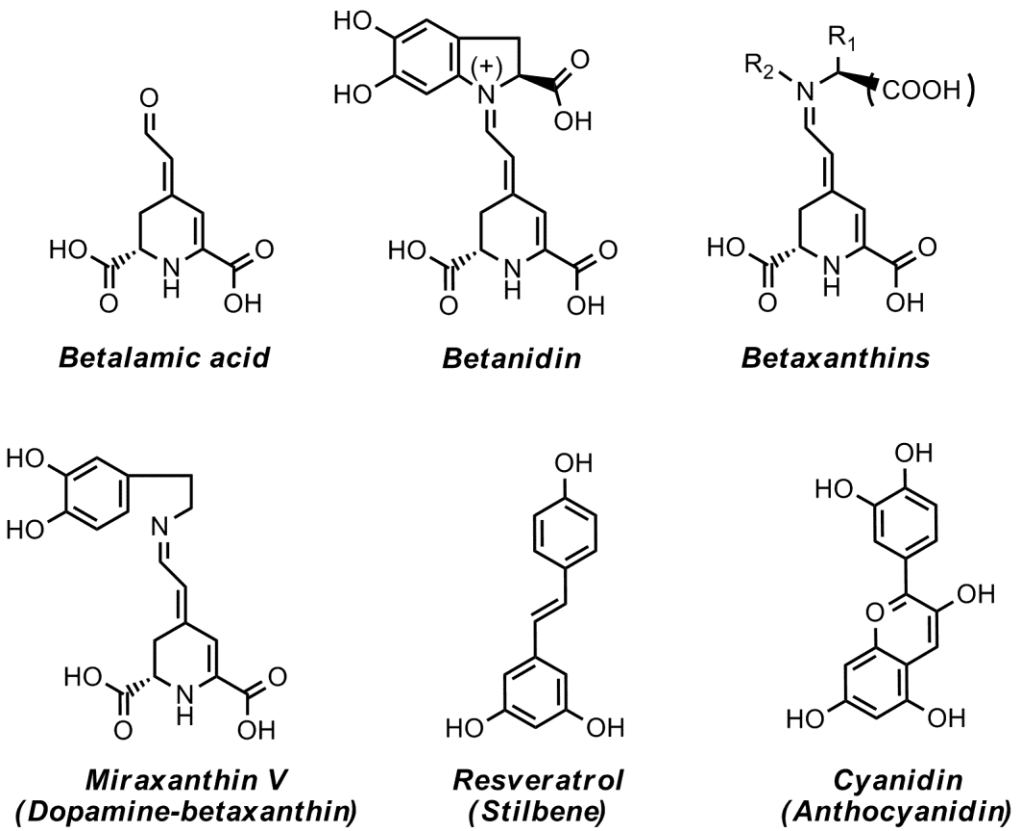

Figure 2. Pictures of the best known sources of betalains: *Opuntia ficus-indica* fruit (A) and *Beta vulgaris* root (B). An encapsulated commercial colorant based on *B. vulgaris* root extracts is also shown (C). A dairy product containing *B. vulgaris* extracts is shown in (D).