

## References

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## Product Information

# Scientific overview Healthberry™ 865

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**Healthberry™ 865 is an innovative, ready-to-use, natural product high in anthocyanins derived from berries. It is standardized towards a constant composition of 30 % anthocyanins, with its main anthocyanins being Cyanidin-3-glucoside (C3G), Delphinidin-3-glucoside (D3G) and Cyanidin-3-rutinoside (C3R).**

### Introduction to anthocyanins

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. Belonging to the group of phytochemicals, they fall under the category of the polyphenols and their subcategory flavonoids. Within the group of the flavonoids anthocyanins stand out, as they are able to form flavylium cations. In respect to their chemical structure, anthocyanins are glycosides or acylglycosides of anthocyanidins that are all polyhydroxy derivatives. In nature six main anthocyanidins are found. They are classified according to the number and location of the hydroxyl and methoxylgroup attached to the flavan nucleus.<sup>1</sup> Besides the hydroxyl and methoxy-groups, sugars like glucose, galactose, arabinose, rhamnose and xylose are attached, either as mono-, di- or tri-saccharides.<sup>2</sup> The six main anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin, of which cyanidin is most widely occurring.<sup>1</sup> In Figure 1 an overview of the different anthocyanidins is presented.

Anthocyanin glycoside	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Cyanidin-3-O-rutinoside	OH	H	rhamnosyl-glucoside
Cyanidin-3-O-glucoside	OH	H	glucoside
Cyanidin-3-O-galactoside	OH	H	galactoside
Cyanidin-3-O-arabinoside	OH	H	arabinoside
Cyanidin-3-O-sambubioside	OH	H	xylosyl-glucoside
Delphinidin-3-O-rutinoside	OH	OH	rhamnosyl-glucoside
Delphinidin-3-O-glucoside	OH	OH	glucoside
Pelargonidin-3-O-glucoside	H	H	glucoside
Peonidin-3-O-glucoside	OMe	H	glucoside
Petunidin-3-O-glucoside	OMe	OH	glucoside
Malvidin-3-O-glucoside	OMe	OMe	glucoside

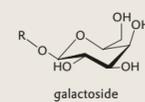
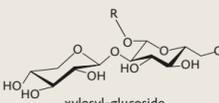
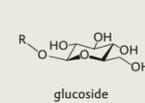
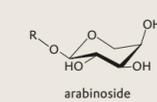
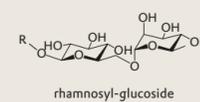
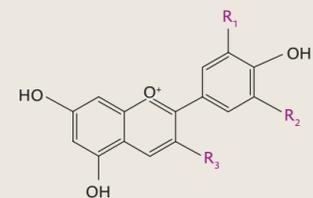


Figure 1. Six main anthocyanins with their glycosides occurring in nature.

Anthocyanins are responsible for the red, blue and purple colors of several fruits and vegetables. Sources with high anthocyanin contents are berries, cherries, red grapes, red wines, red cabbage, eggplant and the black variants of soybean and rice.<sup>1,2</sup> There are several berry based products in the food and dietary supplements market like **Healthberry™ 865** which is a natural product high in anthocyanins derived from berries. It is standardized towards 30% anthocyanins, with its main anthocyanins being Cyanidin-3-glucoside (C3G), Delphinidin-3-glucoside (D3G) and Cyanidin-3-rutinoside (C3R).

#### Scientific support for the positive effects on heart health with Healthberry™ 865

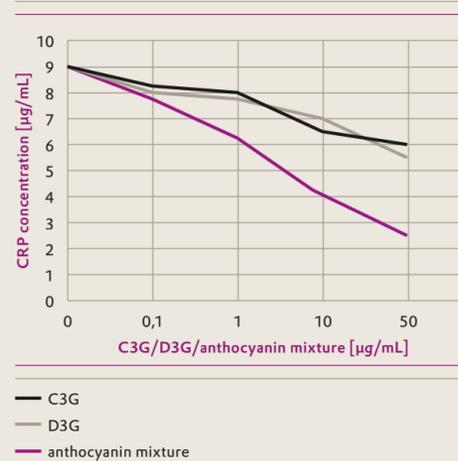
In recent years anthocyanins gained more scientific substantiation for their supportive health effects. Epidemiological studies showed positive correlations between regular flavonoid intakes and the decreased risk of cardiovascular disease (CVD) mortality.<sup>3</sup> An example of a large epidemiological study towards heart health is the Iowa Women Health Study. This 16-years follow-up study with 34,489 CVD-free postmenopausal women showed that intake of food rich in flavonoids was positively associated with reduced risk of CVD and coronary heart disease (CHD) mortality.<sup>4</sup> In 2013 another large follow up study finished, it was an 18-years follow up of 93,600 healthy women between 25–42 years from the Nurses' Health Study (NHS) II. The NHS are the largest and longest running epidemiological studies performed towards lifestyle factors, such as diet, that influence women's health. The recently finished study showed that high anthocyanin intake reduced the risk of myocardial infarction.<sup>5</sup>

CVD is a multi-component pathology with several risk factors that accelerate the disease, such as atherosclerosis, hypertension, hyperlipidemia, inflammation and oxidative stress.<sup>6</sup> Several human intervention studies performed with Healthberry™ 865 as active ingredient of the well-known Medox® product showed positive effects on several of these parameters and thereby promoting heart health.

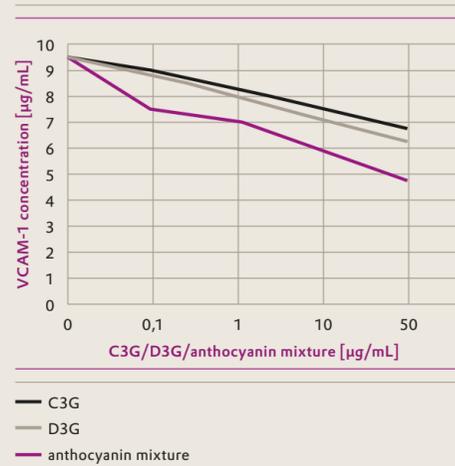
Inflammation is found to be present in CVD pathology. One of the main transcription factors that controls the expression of genes in the inflammatory response is transcription factor Nuclear Factor Kappa B (NF-κB). It is activated by several pro-inflammatory cytokines and oxidative stress.<sup>7</sup> The effect of anthocyanin supplementation on inflammatory regulation was assessed in a parallel-designed placebo-controlled trial (n=120 healthy men and women aged 40–74 years) with anthocyanin supplementation of 300mg/day or a placebo. After 3 weeks of anthocyanin supplementation, significant decreases were found in NF-κB controlled pro-inflammatory cytokine Interleukin 8 (IL-8) and inducer of NF-κB interferon-α (IFN-α). Both were significantly decreased (respectively 45% and 40% compared to

baseline, placebo resp. 20% and 15% decrease from baseline (P>0.050)). Also IL-4 and IL-13 levels (both mediate pro-inflammatory responses and induce NF-κB activation) were significantly decreased after anthocyanin supplementation (resp. 60% and 38% decrease, compared to 4% and 6% in the placebo group (P=0.056 and P=0.089)).<sup>7</sup>

Atherosclerosis is one of the main risk factors of CVD. It is characterized by thickened artery walls, with plaques consisting of white blood cells, dead cells, triglycerides and cholesterol, which narrows the artery and hampers the blood flow. Pro-inflammatory cytokines are involved in the progression of atherosclerosis, especially C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1) and interleukin 1β (IL-1β).<sup>8</sup> The anti-inflammatory effect of anthocyanins was determined in a randomized double-blind trial (n=150 men and women aged 40–65 years with hypercholesterolemia) with anthocyanin supplementation of 320 mg/day or a placebo for 24 weeks. Anthocyanin supplementation decreased significantly the levels of serum high-sensitivity CRP (21.6%, placebo 2.5%, P=0.001), soluble VCAM-1 (12.3%, placebo 0.4%, P=0.005) and plasma IL-1β (12.8%, placebo 1.3%, P=0.019). More in depth research showed that C3G and D3G inhibited the IL-6 and IL-1β-induced CRP production and VCAM-1 secretion in a dose-dependent way. Furthermore, C3G and D3G together showed stronger inhibition, compared to the decreases observed after exposure of the single compounds, see Figure 2A and 2B.<sup>8</sup>



**Figure 2A.** Effects of anthocyanin exposure on C-reactive protein (CRP) expression. HepG2 cells were 2h pretreated with anthocyanins and subsequently for 24h stimulated by 20 ng/mL IL-6 and 10 ng/mL IL-1β. At the end of the treatment period, the conditioned media was collected and CRP levels were measured. Dataset represents three independent experiments.<sup>8</sup>



**Figure 2B.** Effects of anthocyanin exposure on Vascular Cell Adhesion Molecule-1 (VCAM-1) expression. PIEC cells were 2h pretreated with anthocyanins and subsequently for 24h stimulated with 10 µg/mL LPS. At the end of the treatment period, the conditioned media was collected and VCAM-1 levels were measured. Dataset represents three independent experiments.<sup>8</sup>

**Anthocyanins from Healthberry™ 865 are able to interfere in the inflammation cascade by inhibiting NF-κB and C-reactive protein and thereby decrease the plasma concentrations of pro-inflammatory cytokines and other inflammatory mediators. The anthocyanins C3G together exhibit synergistic effects in lowering pro-inflammatory cytokines.**

**Important outcome**

- C3G lowers pro-inflammatory cytokines
- D3G lowers pro-inflammatory cytokines

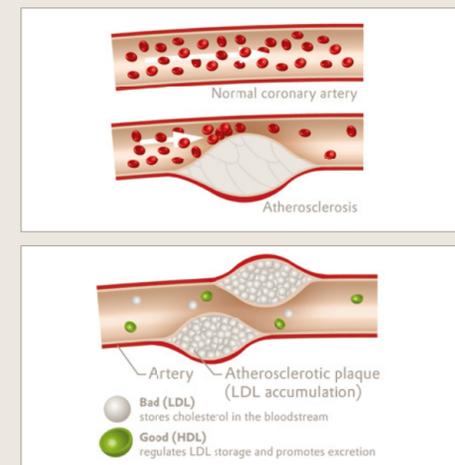
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Clear results that C3G and D3G in combination synergistically lower pro-inflammatory cytokines

High LDL-cholesterol and triglyceride levels are risk factors for the development of CVD, while HDL-cholesterol is inversely related to the risk of developing CVD. LDL-cholesterol delivers cholesterol to the peripheral tissues for synthesis and maintenance of cell membranes. In case more cholesterol is delivered than needed, accumulation in the arteries takes place, and high concentrations can lead to atherosclerotic plaque formation. HDL-cholesterol promotes the transport of cholesterol out of atherosclerotic plaques, back to the liver where it is eliminated from the body as bile salt or biliary cholesterol.<sup>9</sup> Positive effects on cholesterol levels are found after supplementation of Healthberry™ 865. A double-blind randomized

placebo-controlled trial (n=120 dyslipidemic men and women aged 40–65 years) was performed with anthocyanin supplementation of 320 mg/day or a placebo for 12 weeks. Anthocyanin supplementation significantly increased HDL-cholesterol (13.7%, placebo 2.8%, P<0.001) and decreased LDL-cholesterol (13.6%, placebo -0.6% P<0.001) after 12 weeks.<sup>9</sup> These results were supported as well for hypercholesterolemic people. A double-blind randomized placebo controlled trial (n=150 hypercholesterolemic men and women aged 40–65 years) was performed with anthocyanin supplementation of 320 mg/day for 12 weeks. The anthocyanin supplementation led to significant increases in Flow Mediated Dilatation (FMD) (28.4%, 2.2% placebo P<0.05) and significant increases in HDL-cholesterol levels (12.8%, placebo 2%, P<0.05). LDL cholesterol levels were significantly decreased (10%, placebo 0.5%, P<0.05).<sup>10</sup> Also longer exposure of 24 weeks towards anthocyanin supplementation was investigated in hypercholesterolemic men and women, and it led to increases in HDL-cholesterol levels, and decreases in LDL-cholesterol levels.<sup>11</sup> Similar findings were obtained for prehypertensive men. In a small study 27 prehypertensive men aged between 35–51 years were supplemented for 4 weeks 640 mg anthocyanins daily. After this time significant decreases in HDL-cholesterol levels were found (P=0.043), but no change in LDL-cholesterol was observed. Most likely the treatment time was too short to provide a reduction LDL cholesterol.<sup>6</sup>

**The studies show that the anthocyanins from Healthberry™ 865 are able to increase HDL-cholesterol and decrease LDL-cholesterol levels in people with risk factors for the development of cardiovascular disease, and thereby supporting heart health.**



**Figure 3.** Coronary artery affected with atherosclerosis. In the artery plaques with LDL cholesterol are formed. HDL cholesterol has the ability to transport cholesterol out of these plaques and thereby supports cardiovascular health.

#### Indications for other health benefit areas; diabetes, obesity, cancer and eye health

Anthocyanins showed protective effects in other health areas as well; diabetes, obesity, cancer and the support of eye health.<sup>12</sup>

Comparable to CVD, the diabetes type 2 pathology also has several characteristics that accelerate the disease, such as an altered lipid metabolism, hypertriglyceridemia, hyperglycemia, oxidative stress and altered insulin signaling.<sup>13</sup> Most of these characteristics are observed in obesity; therefore obesity is associated with an increased risk of developing diabetes 2. In the development of obesity and diabetes type 2, adipocyte dysfunction is common. This is caused by excess lipid storage in adipocytes which triggers changes; genes for lipid metabolism are expressed differently, and secretions like adipocytokines are lowered compared to a healthy situation.

In a cell-line study with rat adipocytes, exposure to C3G (which is one major anthocyanin in Healthberry™ 865) showed that the gene expression of lipid metabolism and signal transduction-related genes as hormone sensitive lipase and lipolytic activity were upregulated. Also, the secretion of adipocytokines (adiponectin and leptin) was enhanced in rats.<sup>14,15</sup> In a hypercholesterolemia-mediated endothelial dysfunction mice model, it was shown that C3G supplementation reverses the endothelial dysfunction caused by the high-fat cholesterol diet, and simultaneously reverses atherosclerosis.<sup>16</sup>

A study with diabetic mice (that spontaneously become obese upon standard diet) shows that C3G supplementation significantly reduced obesity, fat accumulation in visceral areas and liver tissues and reduced plasma triglyceride levels.<sup>17</sup> Recently, the first human randomized, placebo-controlled double-blind trial towards diabetes (n=58 diabetic patients aged 56–67 years) was performed with Medox® from which Healthberry™ 865 is the active ingredient. The anthocyanin supplementation was done with 320 mg/day or a placebo for 24 weeks. The anthocyanin supplementation led to a significant decrease in LDL-cholesterol (7.9%, -0.6%

placebo, P<0.05), a significant increase in HDL-cholesterol (19.4%, placebo -0.4%, P<0.05) and a significant decrease in triglycerides (23.0%, placebo 3.0%, P<0.05) after 24 weeks. Also the anti-oxidative parameters Total Radical-trapping Antioxidant Parameter (TRAP) and Ferric ion Reducing Antioxidant Power (FRAP) were increased after anthocyanin supplementation, with respectively 24.3% (placebo 2.8%, P<0.05) and 29.8% (placebo 1.9%, P<0.05). Anthocyanin supplementation also affected fasting plasma glucose levels and the Homeostasis Model Assessment for Insulin Resistance index (HOMA-IR index). It showed a significant decrease in fasting plasma glucose levels of 8.5% (placebo 2.8%, P<0.05) and HOMA-IR index by 14% (placebo 1.9%, P<0.05). This shows that Healthberry™ 865 anthocyanin supplementation acts on different parts of the diabetes pathology by improving cholesterol and triglyceride levels, increase the anti-oxidative capacity and prevent insulin resistance.<sup>13</sup>

Anthocyanins occurring in Healthberry™ 865 also show effects in cancer prevention. In two leukemia cell lines the effect of C3G exposure was investigated, and it showed that C3G induced apoptosis and reduced cell proliferation.<sup>18</sup> In malignant glioblastoma cells C3G exposure to glioblastoma cells showed the highest inhibitory potency, and resulted in decreased migration and invasion.<sup>19</sup> This serves as a basis for the potential chemopreventive effects of Healthberry™ 865.

Anthocyanins have been known as well for their effects on eye health. C3G and C3R exposure showed significant effects in the regeneration of rhodopsin, which is involved in the light perception and dark adaptation.<sup>20</sup>

**In conclusion, it is proven that Healthberry™ 865 clearly supports heart health by improving LDL and HDL cholesterol and lowering pro-inflammatory cytokines, and thereby contributes to the prevention of cardiovascular disease. Further health benefits upon Healthberry™ 865 exposure are found in the areas of diabetes, obesity and cancer prevention and eye health.**