

Composition of various parts from Vitis vinifera

The European grapevine (*Vitis vinifera*) contains a range of organic compounds. Various parts of the plant (e.g. grape seeds, grape skin, and grapevine leaf) have a different chemical composition. However, all parts of the plant contain monomeric, oligomeric, and polymeric proanthocyanidins.

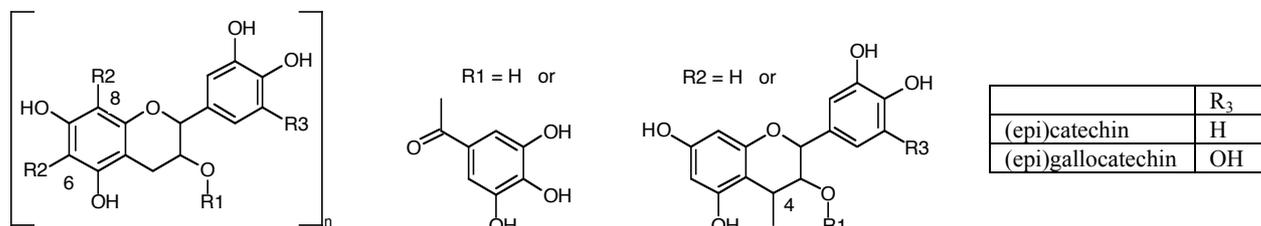


Figure 1 General structure of oligomeric proanthocyanidins.

Proanthocyanidins are oligomers of flavan-3-ol units (catechin, epicatechin, gallocatechin, and epigallocatechin), which are generally coupled through 4→6 and 4→8 links. The most common classes are the procyanidins, which are oligomers of (epi)catechin and their gallic acid esters, and prodelphinidins, which are oligomers of (epi)gallocatechin and their gallic acid esters [Porter (1989) in **Lazarus** et al (1999)]. Grape seeds only contain procyanidins, while other parts (grape skins and stems) also contain prodelphinidins. Therefore, the absence of trihydroxylated flavan-3-ol units (gallocatechin and epigallocatechin) confirms the authenticity of products derived from grape seeds [Vivas et al (2004) and Souquet et al (2000) in **Monagas** et al (2005)].

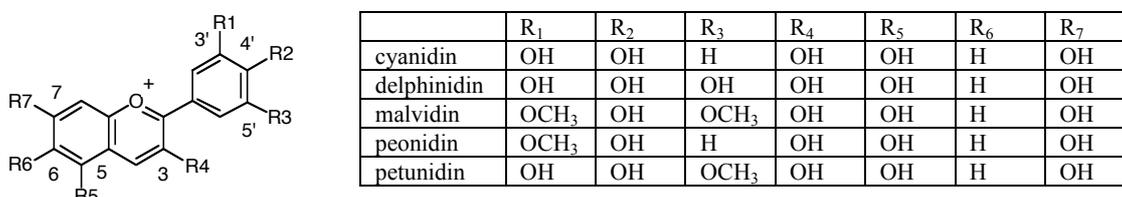


Figure 2 General structure of anthocyanidins.

Anthocyanins are the 3-O-monoglucosides and 3-O-acetylated monoglucosides of the five main anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin). Acetylation may occur at the C-6 position of the glucose molecule by esterification with acetic, p-coumaric, and caffeic acid [Mazza et al (1993) in **Monagas** et al (2006)]. The anthocyanins are water-soluble plant pigments, which are present in the grape skins and grapevine leaves of red cultivars. Glucosides are more abundant than acetylated glucosides. Malvidin-3-O-glucoside is the main pigment in the grape skins, while peonidin-3-O-glucoside, cyanidin-3-O-glucoside, and malvidin-3-O-glucoside are the main pigments in the grapevine leaves [**Monagas** et al (2006)].

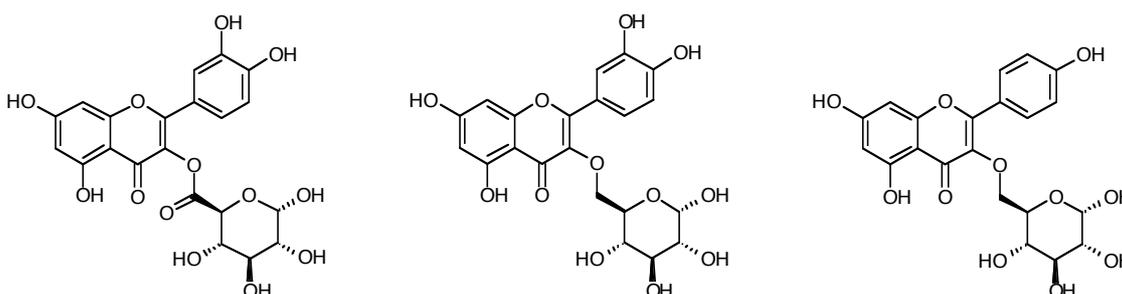


Figure 3 Quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-D-glucoside, and kaempferol-3-O-β-glucoside.

Support for claims regarding the use of grape seed extract *Prevention of UV-induced skin damage*

Flavonols exist as the 3-O-glycosides of myricetin, quercetin, kaempferol, and isorhamnetin. Glucose, galactose, and glucuronic acid are the most common sugar units [Monagas et al (2005) in **Monagas** et al (2006)]. Flavonols are present in grape skins and grapevine leaves of both white and red cultivars. Quercetin derivatives are more abundant in grapevine leaves than kaempferol derivatives. Quercetin-3-O-glucuronide is the most important flavonol, followed by quercetin-3-O-glucoside (isoquercitrin), and kaempferol-3-O-glucoside [**Monagas** et al (2006)].

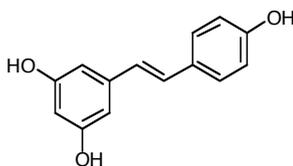


Figure 4 Resveratrol

trans-Resveratrol (3,5,4'-trihydroxystilbene) is an antimicrobial and antifungal compound that is naturally produced by grapevines upon infection. It is accumulated in grapevine leaves and grape skins in response to various fungal organisms, UV radiation, or chemicals [Jeandet et al (1995) and Langcake et al (1976) in **Orea** et al (2001)].

Grape seeds

Grape seeds contain procyanidins. They do not contain prodelphinidins or flavonoid compounds, such as anthocyanins and flavonols [Waterhouse et al (1995) in **Yamakoshi** et al (2002)]. Since 55% of the procyanidins that are extracted from grape seeds contain more than five monomer units, it is concluded that grape seeds contain a mixture of procyanidin monomers, oligomers, and polymers [Prieur et al (1994) in **Yamakoshi** et al (2002)].

Grape seed extracts are generally prepared by extraction with highly polar solvents (e.g. water or mixtures of short chain alcohols and water). Consequently, only water soluble compounds will be present. Grape seed extract is expected to contain mainly procyanidin dimers, trimers, tetramers, and their gallic acid esters. Some (epi)catechin and gallic acid and small amounts of procyanidin pentamers, hexamers, heptamers and their gallic acid esters are also expected to be present.

Grape skins

Grape skins contain procyanidins and prodelphinidins. Anthocyanins are only present in grape skins of red cultivars. They also contain flavonols and *trans*-resveratrol.

Grapevine leaves

Grapevine leaves contain procyanidins and prodelphinidins. Anthocyanins are only present in the grapevine leaves of red cultivars. They also contain flavonols and *trans*-resveratrol. Grapevine leaves also contain various other (non-phenolic) compounds like organic acids (e.g. mainly malic and oxalic acid, but also tartaric acid and traces of citric, fumaric, and succinic acid), carotinoids, and vitamin C [Beck (1997) in **Lardos** et al (2000)].

Grapevine leaf extracts are generally prepared by extraction with highly polar solvents (e.g. water or mixtures of short chain alcohols and water). Consequently, only water-soluble compounds will be present. According to the French Pharmacopoeia monograph, red vine leaf extract ('*extrait de vigne rouge*') should contain not less than 0.2% anthocyanins and not less than 4% polyphenols, which includes oligomeric proanthocyanidins and flavonols.

Support for claims regarding the use of grape seed extract
Prevention of UV-induced skin damage

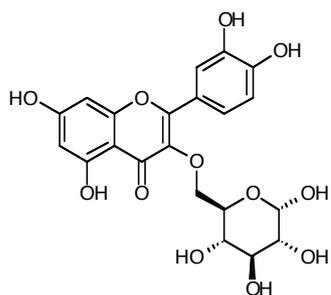


Figure 5 Quercetin-3-O- β -D-glucoside.

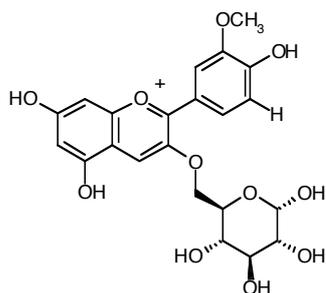


Figure 6 Peonidin 3-O- β -glucoside.

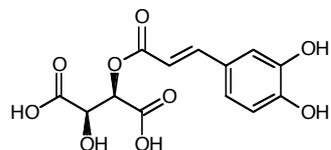


Figure 7 Caffeic acid, tartaric acid ester

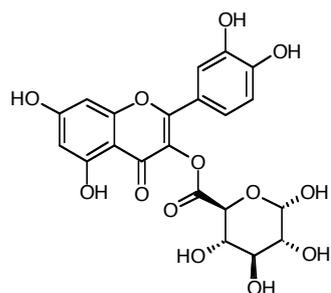


Figure 8 Quercetin-3-O- β -D-glucuronide.

According to the French Pharmacopoeia, identification of dry red vine leaf extract is carried out by thin layer chromatography (TLC), which monitors the following substances: quercetin-3-O- β -D-glucoside, peonidin 3-O- β -glucoside, tartaric acid ester of caffeic acid, and quercetin-3-O- β -D-glucuronide. Tartaric acid ester of caffeic acid unambiguously identifies the botanical source, as it is unique for the *Vitis vinifera* species [Jaworski et al (1987), Oszmianski et al (1990), and Macheix et al (1997) in Lazarus et al (1999)]. Quercetin-3-O- β -D-glucoside and quercetin-3-O- β -D-glucuronide are the major polyphenolic components of grapevine leaves [Monagas et al (2006)]. Peonidin 3-O- β -glucoside, an anthocyanin, is characteristic for the red grape skins and grapevine leaves.

Prevention of UV-induced skin damage by grape seed extract

Inhibition of skin tumour promotion by hydrolysable and condensed tannins

The inhibition of skin tumour promotion by hydrolysable and condensed tannins (gallotannins and polymeric proanthocyanidins, respectively) was investigated in an *in vivo* model. m-Chloro-peroxybenzoic acid was tested for its ability to induce the ornithine decarboxylase marker of skin tumour promotion. The induction is dose dependent and sustained after chronic treatment, while its mechanism is iron dependent. Various hydrolysable and condensed tannins and their monomeric units inhibit the ornithine decarboxylase response to m-chloro-peroxybenzoic acid [Chen et al (1995)]. Additionally, m-chloro-peroxybenzoic acid was tested for its ability to induce DNA synthesis, hydroperoxide production, and tumour promotion in the mouse epidermis *in vivo*. The stimulation of DNA synthesis was similar for m-chloro-peroxybenzoic acid and for 12-O-tetradecanoylphorbol-13-acetate, a known tumour inducer. However, the hydroperoxide response is much weaker for m-chloro-peroxybenzoic acid than for 12-O-tetradecanoylphorbol-13-acetate. Hydrolysable and condensed tannins inhibit both the DNA and hydroperoxide responses, while their monomeric units only inhibit the DNA response. m-Chloro-peroxybenzoic acid is a very weak complete tumour promoter. Hydrolysable and condensed tannins and their monomeric units inhibit complete tumour promotion by m-chloro-peroxybenzoic acid [Chen et al (1996)]. The inhibition of skin tumour promotion by topical application of grape seed polyphenols was studied in an *in vivo* model. Topical application of grape seed polyphenols resulted in a highly significant inhibition of tumour promotion by 12-O-tetra-decanoylphorbol-13-acetate. The observed effects were dose dependent and were evident in terms of a reduction in tumour incidence (35 and 60% inhibition for the 0.5 and 1.5 mg doses, respectively), tumour multiplicity (61 and 83% inhibition for the 0.5 and 1.5 mg doses, respectively), and tumour volume (67 and 87% inhibition for the 0.5 and 1.5 mg doses, respectively). Inhibition of UV radiation-induced peroxidation activity is expected to play a role in the mechanism of action. A structure-activity relationship study showed that with an increase in degree of polymerisation in polyphenol structure, the inhibitory potential towards lipid peroxidation increased. Moreover, procyanidin isomers with a 4–6 linkage showed stronger inhibitory activity than isomers with 4–8 linkage. A sharp increase in the inhibition of epidermal lipid peroxidation was also evident when a gallate group was linked at the 3'-hydroxy position of a procyanidin dimer [Zhao et al (1999)].

Protection of UV-irradiated human skin by a combination of various antioxidants

The short-term photoprotective effects of different antioxidants and their combinations were evaluated *in vivo* in a randomised, double-blind human study. Vitamin C (ascorbic acid), vitamin E (α -tocopherol), and melatonin (*N*-acetyl-5-methoxytryptamine) were topically applied, alone or in combination, 30 min before UV-irradiation of the skin. When applied alone, vitamins showed a modest protective effect, while melatonin showed a dose dependent photoprotective effect. The use of combinations enhanced the photoprotective response. The scavenging of reactive oxygen species and oxygen-derived free radicals as well as the potential sunscreens properties of the applied antioxidants [Dreher et al (1998)]. The photoprotective effect of the oral administration of a combination of lipid and water soluble antioxidants (β -carotene, lycopene, vitamins C and E, selenium, and proanthocyanidins) was investigated *in vivo* in a randomised, double-blind, parallel group, placebo-controlled, clinical study. Although assessment of the light sensitivity (minimal erythema dose, chromametry) of the skin did not show any statistically significant differences, the product was able to slow down the time of the development and grade of UVB-induced erythema. The photoprotective effect might be attributed to a decrease of the UV-induced expression of MMP-1 and MMP-9 [Greul et al (2002)]. The photoprotective effect of the oral administration of either β -carotene or vitamin E was investigated in a clinical study. Neither compound showed any effect on the sensitivity of the skin to UV radiation [McArdle et al (2004)].

Protection of UV-irradiated human skin by oligomeric proanthocyanidins

The anti-inflammatory and skin hydration properties of a dietary supplement and two topical formulations with oligomeric proanthocyanidins were investigated in a randomised clinical trial. Only one group of subjects received dietary supplement (100 mg/day). After 4 weeks, erythema was induced using UV radiation followed by treatment with topical cream or lotion. Both topical formulations led to a significant suppression of erythema formation and the dietary supplement led to an additional slightly stronger suppression. 72 hours after the UV exposure and compared to the controls, erythema was slightly (13.2%) lower in the subjects that had taken dietary supplement [**Hughes-Formella** et al (2007)].

Conclusions regarding the use of grape seed extract to prevent UV-induced skin damage

Studies in *in vivo* models have shown the ability of oligomeric proanthocyanidins to inhibit the formation of skin tumours [**Chen** et al (1995 and 1996) and **Zhao** et al (1999)]. Furthermore, oral administration of a combination of various antioxidants (β -carotene, lycopene, vitamins C and E, selenium, and proanthocyanidins) has been found to reduce UVB-induced erythema in humans [**Greul** et al (2002)]. Since oral administration of β -carotene or vitamin E alone did not exhibit any photoprotective effect [**McArdle** et al (2004)], it would be reasonable to assume that the proanthocyanidins are responsible for the observed photoprotective effect. This assumption has been confirmed by a study involving oligomeric proanthocyanidins [**Hughes-Formella** et al (2006)].

It is concluded that the systemic use of grape seed extract may reduce the extent of skin damage after exposure to UV radiation and prevent the occurrence of skin cancer via various mechanisms. The effect is probably most significant, if administration of the grape seed extract is started prior to exposure to UV radiation. However, some effect may be expected after exposure to radiation.

Taking into account the fact that the grape seed extract capsules will be marketed as a food supplement, the following (non-medical) claims may be allowed in the Netherlands, based on the Dutch so-called KOAG-KAG list (with English translation between brackets):

- 'te lang in de zon' (too much sun exposure)
- 'ter bescherming tegen zon' (for the protection of sun)
- 'beschermt tegen zon' (protects against sun)
- 'beschermt tegen UV-straling' (protects against UV rays)
- 'bij een zongevoelige huid' (in case of a sun sensitive skin)

The most interesting claim has been underlined.

It is very important to include a disclaimer that the product is not intended to be used to replace adequate UV protection (sunscreen or sun block, clothing, parasol, etc.). It should only be used to provide additional protection.

Based on the available literature, it is concluded that the development of a topical dosage form containing grape seed extract (e.g. a cream), may also be useful to prevent or treat erythema that is caused by exposure to UV radiation.

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