Support for claims regarding the use of grape seed extract  
_Treatment of chronic venous insufficiency_

_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, gallicatechin, 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)gallicatechin 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 (gallicatechin 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.
Flavonols exist as the 3-O-glycosides of myricetin, quercetin, kaempherol, 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 kaempherol derivatives. Quercetin-3-O-glucuronide is the most important flavonol, followed by quercetin-3-O-glucoside (isoquercetrin), and kaempherol-3-O-glucoside [Monagas et al (2006)].

\[ \text{Figure 4} \quad \text{Resveratrol} \]

*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 Langeake 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

Treatment of chronic venous insufficiency

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.
Support for claims regarding the use of grape seed extract

_Treatment of chronic venous insufficiency_ (CVI) by grape seed extract

**Physiology of CVI**
CVI includes several clinical manifestations caused by an increased venous pressure and chronic venous stasis of the legs. Varicose veins, calf tenderness or heaviness, pain, ankle or leg oedema, pigmentation of the skin, and ulceration are the most common symptoms [Ibrahim et al (1996) in Petrassi et al (2000)]. Alterations in the venous microcirculation are the main pathogenic factors in CVI [Mani (1992) in Arcangeli (2000)]. Functional and organic alterations primarily affect the cutaneous and subcutaneous capillaries, followed by reduced capillary resistance and increased permeability, resulting in oedema, inflammation, and micro necrosis of the subcutaneous tissues. If the alterations persist, chronic inflammation may develop. Venous pressure then increases and the microcirculatory consequences include morphological changes of the endothelium, functional abnormalities and haemorheological changes, increased capillary permeability, and recruitment of leucocytes [Leu (1994) in Arcangeli (2000)]. Leucocytes are generally regarded as beneficial cells. However, their role as mediators of venous ulceration and other pathological consequences of CVI has been demonstrated [Schmid-Schonbein (1993) in Arcangeli (2000)]. Granulocytes and monocytes adhere to the endothelium and exert several forms of cytotoxicity; they may occlude the capillaries and induce an inflammatory reaction by means of free radical production and proteolytic cleavage. It is well known that activated leucocytes release cytokines, free radicals, proteolytic enzymes, and platelet activating factor [Dormandy (1995) in Arcangeli (2000)]. Among these compounds, free radicals play an important role in the pathogenesis of CVI by modifying endothelial permeability, provoking subcutaneous oedema [McQuid et al (1997) in Arcangeli (2000)], reducing vascular tone [Bharadwaj et al (1997) in Arcangeli (2000)], and inducing leucocyte adhesion and migration [Cominacini et al (1997) in Arcangeli (2000)].

**Studies supporting the treatment of CVI by grape seed extract**
A standardised, purified grape seed extract, containing oligomeric procyanidins (50 or 150 mg), has been registered in France under the brand name Endotelon® for the treatment of functional manifestations of CVI (heavy legs, pain, and restless legs while falling asleep). The efficacy and safety of Endotelon® (150 mg/day for one month) and semi-synthetic diosmine were compared in a double-blind study in 50 patients with symptoms of CVI. These patients were recruited from a gynaecology-obstetrics outpatient clinic. Both subjective and objective criteria were used to evaluate the drugs compared. Both drugs were effective in peripheral venous insufficiency but there was a statistically significant advantage in favour of Endotelon® with regard to rapidity and duration of action. Neither drug was associated with significant toxicity. Side-effects were uncommon and never serious. Both drugs produced transient epigastric discomfort in some patients; nausea and vertigo were seen with Endotelon®. Treatment never had to be discontinued because of side-effects. No contra-indications for the use of the drug in pregnancy were found [Delacroix et al (1981)]. In another placebo-controlled double-blind study of 92 patients, Endotelon® (300 mg/day for 28 days) was effective in treating 75% of patients suffering from peripheral venous insufficiency, while 41% of patients receiving a placebo improved. Parameters such as pain, paraesthesias, nocturnal cramps and oedema were reduced by more than 50% [Thebaut et al (1985)].

**Studies supporting the treatment of CVI by pine bark extract**
French maritime pine bark extract (Pycnogenol®) has also been used for the treatment of CVI. The qualitative and quantitative content of monomeric and oligomeric polyphenols, which are the active ingredients, is comparable in pine bark and grape seed extracts. However, pine bark extract also contains trace amounts of other compounds like taxifolin and phenolic acids, such as ferulic, caffeic, protocatechuic, p-hydroxybenzoic, and vanillic acid [Rohdewald (1998) in Koch (2002)]. In an open, controlled, comparative study, 40 patients with diagnosed CVI were treated either
Support for claims regarding the use of grape seed extract

*Treatment of chronic venous insufficiency*

with horse chestnut seed extract (Venostatin®, 600 mg/day) or French maritime pine bark extract (Pycnogenol®, 360 mg/day) over a period of 4 weeks. Pycnogenol® significantly reduced the circumference of the lower limbs and significantly improved subjective symptoms. Moreover, Pycnogenol® significantly decreased cholesterol and LDL values in the blood, whereas HDL remained unaffected. Venostasin® did not significantly reduce the circumference of the lower limbs and marginally improved symptoms. Venostasin® had no influence on the determined lipid values. Both medications were equally well tolerated [Koch (2002)]. Efficacy of Pycnogenol® in the treatment of CVI was investigated. The study consisted of a double-blind phase, in which 20 patients were recruited and randomly treated with placebo or Pycnogenol® (300 mg/day for two months), and an open phase, in which another 20 patients were treated with Pycnogenol® at the same dose schedule. In total, 40 patients were enrolled; 30 were treated with Pycnogenol® and 10 with placebo. Pycnogenol® significantly improved the legs' heaviness and subcutaneous oedema; the venous pressure was also significantly reduced by the Pycnogenol® treatment, thus adding further clinical evidence to its therapeutic efficacy in patients with CVI. Pycnogenol® was effective, probably by either stabilizing the collagenous subendothelial basal membrane or scavenging the free radicals, or by a combination of these activities. Clinically, capillary leakage, perivascular inflammation and subcutaneous oedema were all reduced. The safety of use of Pycnogenol® was demonstrated by the lack of side effects or changes in blood biochemistry and haematological parameters. Pycnogenol® could therefore be recommended both for prevention and treatment of CVI and related vено-capillary disturbances [Petrassi et al (2000)]. Forty patients with CVI and varices of the legs were selected and double-blindly randomly assigned to a treatment with Pycnogenol® (300 mg/day or a placebo for 2 months), according to a double-blind experimental design. The Pycnogenol® treatment induced a significant reduction in subcutaneous oedema as well as heaviness and pain in the legs, on both after 30 and 60 days, the evaluation time periods. Approximately 60% of patients treated with Pycnogenol® experienced a complete disappearance of oedema (the most rapidly disappearing symptom) and pain at the end of the treatment, while almost all the patients reported a reduction in leg heaviness which disappeared in approximately 33% of patients. These changes were statistically significant. No effect was observed in the placebo-treated subjects. No effect on the venous blood flow was observed in either of the experimental groups [Arcangeli (2000)].

**Studies supporting the treatment of CVI by red grapevine leaf extract**

Some randomised, double-blind, placebo-controlled studies were performed to investigate the efficacy and safety of a standardised red vine leaf extract (AS 195®) for the treatment of CVI [Kiesewetter et al (2000) and Kalus et al (2004)]. One of the studies specifically focussed on an improvement of the cutaneous microcirculation and oxygen supply [Kalus et al (2004)]. Some observational clinical studies were also performed [Schäfer et al (2003) and Monsieur et al (2006)]. One of the studies focussed on the oedema protective properties [Schäfer et al (2003)]. AS 195® contains a variety of flavonols, including its main constituents, quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-D-glucoside, and kaempferol-3-O-β-glucoside [Kalus et al (2004)]. Since the active ingredients in grape seeds extracts (oligomeric procyanidins) and red vine leaf extract (flavonols) are different, results from studies on red vine leaf extract may not be valid for grape seed extract. However, structural similarities between both groups of compounds (oligomeric procyanidins and flavonols) imply that their mechanism of action might be comparable.

**Activity of polyphenolic compounds for the treatment of CVI**

Support for claims regarding the use of grape seed extract

_Treatment of chronic venous insufficiency_

The activity of oligomeric procyanidins (Pycnogenol®) for the treatment of CVI may be based on the following three basic functions: scavenging of free radicals, binding to particular proteins, and stimulation of nitric oxide synthesis [Rohdewald (1998) in Koch (2002)].

Red wines and grapes exhibit endothelium-dependent relaxation of blood vessels via enhanced generation and/or increased biological activity of NO. The degree of vasodilation is correlated to the content and type of phenols. The ability of wines to act both as vasodilators _ex vivo_ and as antioxidants _in vitro_ is strongly correlated with the phenolic content of wines; while antioxidant activity is associated with different classes of phenols (gallic acid, resveratrol, and catechins), vasodilation activity is only correlated with the total content of anthocyanins [Waterhouse (2002) in Dell'Agli et al (2004)]. Anthocyanin enriched fractions and oligomeric proanthocyanidins (dimers, trimers and tetramers) are the active compounds.


Red wine affects the formation of other mediators of vascular tone, such as endothelium-derived hyperpolarising factor [Ndiiaye et al (2003) in Dell'Agli et al (2004)] and prostacyclin [Derek et al (1997) in Dell'Agli et al (2004)]. In addition, the synthesis of a potent vasoconstrictor such as endothelin-1 is reduced by red wine in bovine aortic endothelial cells [Corder et al (2001) in Dell'Agli et al (2004)].

The mechanisms underlying NO-dependent vasorelaxation include an increase in intracellular calcium by enhancing extracellular calcium entry and by increasing calcium mobilisation from intracellular stores [de Aetano et al (2001) in Dell'Agli et al (2004)].


Support for claims regarding the use of grape seed extract

Treatment of chronic venous insufficiency

The in vitro free radical scavenging and in vivo anti-inflammatory activities of oligomeric pro-cyanidines (Pycnogenol®) were found to be closely correlated (r = 0.992), which may account for the anti-inflammatory action in patients with CVI [Petrassi et al (2000)].

Oligomeric procyanidines (Pycnogenol®) counteracts oedema by sealing leaky capillaries, since procyanidins have a high affinity to proteins [Rohdewald et al (1998) in Koch (2002)]. It has been demonstrated in vitro that collagen is protected from the degradation by collagenase when collagen fibres had been pre-treated with catechin [Kuttan et al (1981) in Koch (2002)]. The same has been found for elastine, which is rendered resistant towards elastase degradation after pre-treatment with oligomeric procyanidins [Tixier et al (1984) in Koch (2002)]. In vitro, catechin and procyanidins specifically bind to elastine, significantly reducing its degradation by either porcine pancreatic elastase or human leukocyte elastase [Tixier et al (1984) in Petrassi et al (2000)].


Anthocyanins are absorbed in their glycosilated form. Their bioavailability is limited. They are absorbed at the gastro-intestinal level through a carrier-mediated system, since they are polar and bulky molecules.


Conclusions regarding the use of grape seed extract for the treatment of CVI

Studies have shown that grape seed, pine bark, and red grapevine leaf extract are all suitable for the treatment CVI. Furthermore, it is clear that in all cases the activity towards CVI should be attributed to (various) polyphenolic compounds. Consequently, it is concluded that a standardised grape seed extract (with defined content of oligomeric polyphenols) is suitable to treat CVI.

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):

- 'bij ontzierende aderen' (in case of marred veins)
- 'bij vermoeide benen (en voeten)' (in case of heaviness in the legs)
- 'zware benen' (heavy legs)
Support for claims regarding the use of grape seed extract
*Treatment of chronic venous insufficiency*

*Literature*

**Dell’Agli** M, Buscialà A, Bosisio E (2004)
Vascular effects of wine polyphenols

**Arcangeli** P (2000)
Pycnogenol® in chronic venous insufficiency
Fitoterapia 71, 236-244.

**Delacroix** P (1981)
Double blind trial of Endotelon® in chronic venous insufficiency

Improvement of cutaneous microcirculation and oxygen supply in patients with chronic venous insufficiency by orally administered extract of red vine leaves AS 195
Drugs R D 5, 63-71.

Efficacy of orally administered extract of red vine leaf AS 195 (folia vitis viniferae) in chronic venous insufficiency (stages I-II): a randomized, double-blind, placebo-controlled trial

**Koch** R (2002)
Comparative study of Venostatin® and Pycnogenol® in chronic venous insufficiency
Phytother Res 16, S1-S5.

**Lardos** A, Kreuter MH (2000)
Red vine leaf
Unpublished paper from Flachsmann AG, Zurich, Switzerland.

**Lazarus** SA, Adamson GE, Hammerstone JF, Schmitz HH (1999)
High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages

Quality assessment of commercial dietary antioxidant products from *Viti vinifera* L. grape seeds
Nutr Cancer 53, 244-254.

Commercial dietary ingredients from *Vitis vinifera* L. leaves and grape skins: antioxidant and chemical characterization
J Agri Food Chem 54, 319-327.

Efficacy of red vine leaf extract AS 195 in chronic venous insufficiency
Schweiz Rundsch Med Prax 95, 187-190.
Support for claims regarding the use of grape seed extract

*Treatment of chronic venous insufficiency*

**Orea** JM, Montero C, Jiménez JB, Ureña AG (2001)
Analysis of *trans*-resveratrol by laser desorption coupled with resonant ionization spectrometry. Application to *trans*-resveratrol content in vine leaves and grape skin
Anal Chem 73, 5921-5929.

**Petrassi** C, Mastromarino A, Spartera C (2000)
Pycnogenol® in chronic venous insufficiency
Phytomedicine 7, 383-388.

Oedema protective properties of red vine leaf extract AS 195 (*Folia vitis viniferae*) in the treatment of chronic venous insufficiency: a 6-week observational clinical trial

**Thebaut** JF, Thebaut P, Vin F (1985)
Study of Endotelon® in functional manifestations of peripheral venous insufficiency
Gaz Med 92, 96-100.

Safety evaluation of proanthocyanidin-rich extract from grape seeds