Grape seed

Vitis vinifera L. belongs to the Vitaceae family. Common name grapevine or grape; it is a perennial woody vine with stems up to 35 m long although in cultivation, it is usually pruned to 1–3 m. Leaves are thin, circular to circular-ovate, 5–23 cm broad, margins dentate or jagged, 4–5-lobed, glabrous opaque-green above, tomentose gray beneath.

Flowers are numerous, in dense clusters opposite to leaves. The fruits – called grapes – are small, pulpy berries, 6-12 mm in diameter, with different shapes: globose, oblong, etc. and colors varying with the variety: green, red, purplish-black, etc. The number of seeds also varies between 2 and 4.

A grape is composed of:

- **Petiole**: grape extremity inserted in the woody vienshoot.
- **Skin**: thin layer enveloping the grape
- **Pulp**: fleshy part of the fruit containing the must or juice.
- **Seeds**: inside the grapes, with a hard woody external layer; seeds are different for the different grapevine varieties. A single grape has usually 4 seeds, because they are born from two ovaries, each with two ovules. However, since fecundation may be incomplete, the number of seeds ranges from 1 to 4.

![Fig.1. Parts of a grape (hollejo: skin; pulpa: pulp or flesh; semilla: seed).](image)

Grapevine is native to the Mediterranean area and Asia Minor; at present it is cultivated worldwide.

Grape seed extract is produced from the seeds of *Vitis vinifera*. 
CHEMISTRY

Polyphenols
Most abundant phenolics in grape seeds are: chatechins (catechin, epicatechin and proanthocyanidin) and polymers made of them, in particular: gallic acid, monomer flavan-3-ol-catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin 3-O-gallate, dimmers, trimers and higher-polymerized proanthocyanidins.

Proteins
Proteins in grape seeds (7-10%) are composed of the following amino acids: arginine, cisteine, leucine (11.4%), valine and phenylalanine.

Triglycerides
Triglycerides in grape seeds (6-20%) are composed of the following fatty acids: palmitic acid, stearic acid, oleic acid (37%) and linoleic acid (55%).

Unsaponifiable
Phytosterols (0.5-1%): β-sitosterol.
Phytosterols are vegetable sterols. Their chemical structure and the function are similar to those of cholesterol, a sterol of animal origin.
Phospholipids
Phosphatidylserine, phosphatidylinositol, lecithin, cephalin, cerebrosides and phosphatidic acid. Phospholipids are ionic lipids composed of a cholesterol molecule and two fatty acids (1,2-diacylglycerol) with a phosphate group. The phosphate group is attached – by means of a phosphodiester bond – to other often nitrogen-containing group (choline, serine, ethanolamine). Usually, the molecule is electrically charged. Cell membranes consist in phospholipids bilayers. The best studied phospholipids are: phosphatidylinositol, phosphatidic acid and phosphatidylserine. Phospholipids also account for about 50% of lecithin.

Vitamin E
Vitamin E, also called α-tocopherol, is a liposoluble vitamin with antioxidant activity.

TRADITIONAL USES

*Vitis vinifera* is one of the most extensively used species since the times of most ancient civilizations existing of the Earth. Mentions to vineyards and wine can be found in Egyptian hieroglyphics dated 2400 B.C. and in a number of Bible verses. Hippocrates, Theophrastus, Galen, Dioscorides and Pliny mentioned medicinal properties of wine. They recommended red wine as a tonic and astringent, and white wine as a diuretic. Oil extracted from grape seeds is used a dietary complement. In southern Africa, grape seed oil is used as a laxative, antacid, chalagogue and wound healing medicine.

COSMETIC PROPERTIES

Antioxidant activity
Proanthocyanidins, also known as procyanidins, are powerful free radical scavengers and lipid peroxidation inhibitors. The scavenging activity of flavan-3,4-diol – one of the basic structures of proanthocyanidins – has been demonstrated in vitro with different biochemical models, which reproduced the conditions of certain pathologies such as ischemia, inflammation or diabetes (Bombardelli, E. & Morazzoni, P., 1995).

Several studies have demonstrated the antioxidant capacity of the proanthocyanidins in *V. vinifera* seeds. In one of such experiments, proanthocyanidins were tested for superoxide radical scavenging and lipid peroxidation inhibition in rat-liver microsomes. The effects were compared with those observed for the proanthocyanidins from *Cupressus sempervirens*, the anthocyanins from *V. vinifera*, *Vaccinium myrtillus* and *Ribes nigrum*. The proanthocyanidins from *V. vinifera* were the most active superoxide radical scavenger (IC$_{50}$=10μg/ml) and lipid peroxidation inhibitor (IC$_{50}$=16μg/ml) (Bombardelli, E. & Morazzoni, P., 1995).
In a further study involving a different experimental model, grape-seed proanthocyanidins dose-dependently inhibited iron-promoted lipid peroxidation in phosphatidylcholine liposomes (PCL) (IC_{50}=2.5 μmol/l); such inhibition was stronger than that produced by the reference substance, monomeric catechin (IC_{50}=50 μmol/l). In a different experimental model involving ultrasound-induced lipid peroxidation in PCL, where the different phases of the oxidative process could be discriminated, proanthocyanidins remarkably reduced the production of conjugated dienes during the induction phase (IC_{50}=0.1 μmol/l). The later effect was probably due to their direct interaction with OH• radicals as it was demonstrated by electron spin resonance (ESR) (Bombardelli, E. & Morazzoni, P., 1995).

Even during the propagation phase, characterized by a progressive increase of conjugated dienes, incubation with proanthocyanidins was found to dose-dependently inhibit the production of conjugated dienes (IC_{50}=0.05 μmol/l); the activity of α-tocopherol was remarkably weaker in both phases (Bombardelli, E. & Morazzoni, P., 1995).

Table 1 shows the antioxidant activity of grape seed proanthocyanidins, as compared with that of α-tocopherol, in the different experimental models.

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Proanthocyanidins IC_{50} (μmol/l)</th>
<th>α-Tocopherol IC_{50} (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron-promoted lipid peroxidation</td>
<td>2.5</td>
<td>Non detectable</td>
</tr>
<tr>
<td>Ultrasound-induced lipid peroxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) General antioxidant activity</td>
<td>0.1</td>
<td>Non detectable</td>
</tr>
<tr>
<td>b) Induction phase</td>
<td>0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>c) Propagation phase</td>
<td>0.05</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Table 1. Antioxidant activity of proanthocyanidins (Bombardelli, E. & Morazzoni, P., 1995).

Additionally, proanthocyanidins non-competitively inhibited the xanthine-oxidase enzyme, which promotes superoxide anion production with a similar intensity (IC_{50}=2.4 μmol/l) to that of the reference compound alopurinol (Bombardelli, E., & Morazzoni, P., 1995).

Torres, J.L. et al. (2002) and Shi, J. et al. (2003) also demonstrated antioxidant activity for grape seed proanthocyanidins. The later study mentions that the antioxidant power of grape seed proanthocyanidins is 20 times greater than that of vitamin E and 50 times greater than that of vitamin C.

Proanthocyanidins may be included in cosmetic-medicinal treatments for aged skin or skin damaged by external deleterious agents, such as: UV, free radicals, adverse weather, etc. Their antioxidant effects greatly contribute to stop skin damage. Proanthocyanidins have been observed to inhibit several groups of enzymes, such as elastases, collagenases, hyaluronidases and β-glucuronidases. All these enzymes are involved in the degradation of structural components in the extra-cellular matrix (collagen, elastin and hyaluronic acid) and in the vascular endothelium (Alonso, J., 2004).

Therefore, grape seed extract is of great use to formulate cosmetic products for the protection of skin and hair against oxidative processes.
Anti-inflammatory activity
Grape seed phenolic compounds inhibit enzymatic systems involved in the production of free radicals and associated to inflammatory responses. Furthermore, procyanidins inhibit the activation the hyaluronidase enzyme, which degrades proteoglycans and affects a number of tissues during inflammation (Shi, J. et al., 2003).

Li, W.G. et al. (2001) investigated the anti-inflammatory effect and action mechanism of proanthocyanidins from grape seeds. They used croton oil-induced ear swelling in mice and carrageenan-induced hind paw edema in rats. Their results lead them to the conclusion that proanthocyanidins have anti-inflammatory effects on experimental inflammation. Such an anti-inflammatory action was due to their free radical scavenging activity and inhibitory activity on lipid peroxidation and formation of inflammatory cytokines.

Therefore, grape seed extract is recommended to formulate cosmetic products with anti-irritant activity.

Vasoprotective activity
During inflammation-associated activation of leukocytes, intracellular degranulation occurs, due to the secretion of lysosomal proteases and a number of active oxygen species. Abnormally large amounts of proteases and active oxygen species, damage elastic fibers and endothelial cell membranes, respectively. The powerful actions of proanthocyanidins as free radical scavengers and lipid peroxidation inhibitors are the main factors underlying their vasoprotective effects and strengthen their anti-protease properties (Bombardelli, E., & Morazzoni, P., 1995).

Proanthocyanidins have been shown to inhibit proteolytic enzymes such as collagenase, elastase, hyaluronidase and β-glucuronidase, all of them involved in the degradation of collagen, elastin and hyaluronic acid, the main structural components in the extra-vascular matrix. Proanthocyanidins scavenging activity also contributes to preserve the integrity of the vascular endothelium (Bombardelli, E., & Morazzoni, P., 1995).

Table 2 shows the enzyme-inhibitory activity of proanthocyanidins.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Proanthocyanidins IC&lt;sub&gt;50&lt;/sub&gt; (μmol/l)</th>
<th>Enzyme</th>
<th>Procyanidins IC&lt;sub&gt;50&lt;/sub&gt; (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine-oxidase</td>
<td>2.4</td>
<td>Hyaluronidase</td>
<td>80.0</td>
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<tr>
<td>Elastase</td>
<td>4.2</td>
<td>β-Glucuronidase</td>
<td>1.1</td>
</tr>
<tr>
<td>Collagenase</td>
<td>38.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Enzyme inhibitory activity of proanthocyanidins (Bombardelli, E. & Morazzoni, P., 1995).

Proanthocyanidins are especially recommended for blood-circulation insufficiency or related conditions, where endothelial walls are at risk of tearing. The vasoprotective actions of proanthocyanidins are partly due to their activity on the intima layer: They attach to glycosaminoglycans thus stabilizing collagen fibers and consequently reducing capillary permeability. Such a vasoprotective activity was tested and verified in clinical studies using capillary dynamometry with neuropathy patients, as well as in experimental studies involving intra-dermal injections of histamine, bradykinin and collagenase with several experimental models (Alonso, J., 2004).

In one of these studies, oral daily 150 mg proanthocyanidins improved venous tone, as measured by gas plestimography, in patients with enlarged varicose-veins (Alonso, J., 2004).

Thus, proanthocyanidins are vein-active compounds beneficial to treat blood-circulation disorders, such as capillary fragility and chronic venous insufficiency.
Antimicrobial activity
Palma, M. et al. (1999) carried out a study, where grape seeds were subjected to sequential supercritical fluid extraction. By increasing the polarity of the supercritical fluid using methanol as a modifier of CO\(_2\), it was possible to fractionate the extracted compounds. Two fractions were obtained; the first (fraction A), which was obtained with pure CO\(_2\), contained mainly fatty acids, aliphatic aldehydes, and sterols. The second fraction (fraction B), obtained with methanol-modified CO\(_2\), had phenolic compounds, mainly catechin, epicatechin, and gallic acid. The fractions were bioassayed. Antimicrobial activities were checked on human pathogens, and a high degree of activity was obtained with both fractions although fraction A showed stronger effects.

Table 3 shows the antimicrobial effects of fractions A and B of grape-seed extract and of catechin and epicatechin against human pathogens.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fraction A</th>
<th>Fraction B</th>
<th>Catechin</th>
<th>Epicatechin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1500 1000 500</td>
<td>1500 1000 500</td>
<td>1500 1000 500</td>
<td>1500 1000 500</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+ - -</td>
<td>+ + -</td>
<td>- - -</td>
<td>+ + -</td>
</tr>
<tr>
<td>Staphylococcus coagulans niger</td>
<td>++ ++ +</td>
<td>++ + +</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>++ + -</td>
<td>+ + -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Escherichia cloacae</td>
<td>++ ++ +</td>
<td>+ + + +</td>
<td>- - -</td>
<td>- - - +</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
</tr>
</tbody>
</table>

Table 3. Antimicrobial effects of fraction A, fraction B, catechin and epicatechin against human pathogens.

"4mm disc impregnated with selected concentrations of fraction A or fraction B.
+++, strong inhibition (diameter > 15mm); +, inhibition (15mm > diameter < 10mm); -, no inhibition (diameter < 10mm).

Table 3 shows that fraction A exerted the strongest antimicrobial action against S. coagulans niger, E.cloacae, C. freundii and E.coli. The later bacteria were sensitive to all tested concentrations of fraction A, even the lowest one. S.aureus was only moderately sensitive to the highest concentration. B.cereus and the fungus A.flavus were resistant to fraction A at every concentration. In the literature, there are reports concerning the modulation properties of sterols over the bioactivities of other compounds, so it may be possible that sterols produced the effects shown by fraction A. The antimicrobial action of fraction B was weaker than that of fraction A, with maximum concentrations displaying only moderate activity. However, the profile of activity was more homogeneous than that of fraction A. No activity was found for catechin except for a moderate activity against E.coli. Since the activity profile of epicatechin was similar to that of fraction B, it would be possible that the antibacterial action of the later fraction is mainly due to its epicatechin content.

Thus, grape seed extract is of great use to formulate cosmetic products with disinfectant or purifying activity.
Hair growth stimulating activity
Takahashi, T. et al. (1998) carried out an extensive search of natural products with hair-growth stimulating activity.

They found that proanthocyanidins extracted from grape seeds promoted proliferation of hair follicle cells isolated from mice by about 230% relative to controls (100%) with a 3 μM concentration in a 5 days cell culture. Minoxidil (vasodilator substance used to treat androgenic alopecia) was less effective, with a 400 μM concentration inducing 160% proliferation.

Proanthocyanidins also showed remarkable hair-cycle-converting activity from the telogen phase to the anagen phase in C3H mice in vivo test systems. The profile of the active fraction of the proanthocyanidins was elucidated by thiolytic degradation and tannase hydrolysis. We found that the constitutive monomers were epicatechin and catechin; and that the degree of polymerization was 3.5.

These authors observed that cells in the hair follicles, cultured in a proanthocyanidin-containing medium, became globose with the characteristic aspect of undifferentiated cells. This finding suggested that the action mechanism of proanthocyanidins involved preventing cell differentiation and keeping the cells in the growing stage. It is assumed that the growth-promoting effects of proanthocyanidins on the outer root sheath cells switch the bulb region to the growing phase by some mechanism, causing the follicular hair cycle to convert from telogen phase to anagen phase. Minoxidil also had growth-promoting effects on hair follicle cells, although weaker than those of proanthocyanidins.

Interestingly, epicatechin – a monomer of proanthocyanidins – showed no hair-growth stimulation and hair-growth seemed to depend on the oligomeric structure of proanthocyanidins. The authors proposed that the physiological action mechanism of proanthocyanidins involves the modulation of certain signal transduction cascades.

All these findings support the use of proanthocyanidins – hence, the use of grape seed extract – to treat androgenic alopecia.

Immunostimulatory activity
Nair, N. et al. (2002) carried out a study with a proanthocyanidin-containing grape seed extract, to evaluate its immunostimulatory activity. Their results showed that grape seed extract significantly induced the transcription of interferon-gamma (INF-γ) mRNA. INF-γ is a cytokine produced by Th1 leukocytes, which also produce IL-2 and stimulate cell-mediated immunity (unspecific immune system). These results were demonstrated by reverse transcription-PCR. Grape seed extract had no effect on IL-6, a cytokine derived from Th2 leukocytes, which also regulate the activity of the Specific Immune System. We observed a concomitant increase in the number of cells with intracytoplasmic INF-γ as well as the synthesis and secretion of INF-γ. Our results support the hypothesis that grape seed extract has immunostimulatory activity.

The functions of cytokines are:

- to eliminate rests of dead cells or other substances foreign to the organism, so that cell regeneration factors can exert their functions
- to activate cell regeneration factors

Such an immunostimulatory activity, makes grape seed extract recommendable for acne-treatments, because it stimulates the elimination of dead cells and microorganisms (pus) and its cell-regeneration effects are beneficial for acne as well as for sensitive/irritated skin.

Furthermore, age is known to slow-down immune activation, which results in slower cell regeneration. Grape seed extract is thus beneficial for aged skin.
Finally, we would like to mention a monograph included in *Plants preparations used as ingredients of cosmetic products* (Council of Europe Publishing, 1994) on *V.vinifera* fruits extract, *V.vinifera* seed oil and *V.vinifera* leaves extract, where the following cosmetic properties are attributed to these preparations:

- fruit: colorant (anthocyanins), protective, moisturizing
- seeds: emollient, protective, anti-aging
- leaves: tonic, astringent, refreshing (tannins) anti-aging

Maximum recommended concentration is 15% for lotions, creams, aged-skin care gel and hair products.

Other possible effects are:
- polyphenols: free radical scavengers, micro-circulation protective, anti-irritant, antioxidant
- unsaponifiable: granulation-promoting agent

**COSMETIC APPLICATIONS**

<table>
<thead>
<tr>
<th>Action</th>
<th>Active</th>
<th>Cosmetic Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant</td>
<td>Proanthocyanidins</td>
<td>- Anti-aging</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Photo-protection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Hair color protection</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Proanthocyanidins</td>
<td>- Anti-irritant</td>
</tr>
<tr>
<td>Vasoprotective</td>
<td>Proanthocyanidins</td>
<td>- Blood circulation stimulation</td>
</tr>
<tr>
<td>Antimicrobial</td>
<td>Fatty acids</td>
<td>- Disinfectant</td>
</tr>
<tr>
<td></td>
<td>Aliphatic aldehydes</td>
<td>- Purifying</td>
</tr>
<tr>
<td></td>
<td>Sterols</td>
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</tr>
<tr>
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<td>Phenolic compounds</td>
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</tr>
<tr>
<td>Hair-growth stimulatory</td>
<td>Proanthocyanidins</td>
<td>- Anti-alopecia</td>
</tr>
<tr>
<td>Immunostimulatory</td>
<td>Proanthocyanidins</td>
<td>- Anti-acne</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Anti-irritant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Anti-ageing</td>
</tr>
</tbody>
</table>

**RECOMMENDED DOSE**

The recommended dose is between 0.5% and 5.0%.
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Palma M, Taylor LT, Varela RM, Cutler SJ, Cutler HG. Fractional extraction of compounds from grape seeds by supercritical fluid extraction and analysis for antimicrobial and agrochemical activities. J Agric Food Chem, 1999; 47(12):5044-5048.

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