



15 July 2010
EMA/HMPC/16633/2009
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Vitis vinifera* L., folium

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Vitis vinifera</i> L., folium
Herbal preparation(s)	Herbal substance - Not applicable Herbal preparations: - Comminuted dried leaves as herbal tea (TU) - Powdered herbal substance (TU) - Soft extract (2.5-4:1; water) (TU) - Dry extract (4-6:1; water) (WEU)
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use (TU). Herbal preparation in solid dosage forms for oral use (WEU and TU). Herbal preparation in semi-solid dosage forms for cutaneous use (TU).
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1. Introduction

The aim of this report is to assess the preclinical and clinical available data on *Vitis viniferae folium* for preparing a Community herbal monograph and Community List entry. This report is based on the documentation provided by the European Medicines Agency (EMA) completed by additional searches and information taken from recent international literature on *Vitis viniferae folium*.

Bibliographic searches have been performed in MEDLINE (1966-2008); EMBASE (1974-2008). The search terms were: Plant extracts; Plant leaves; Red vine leaf extract; Roter Weinlaubextrakt; Extract Red vine / wine Leaf / Leaves *Vitis vinifera*; Folia *Vitis vinifera*.

Only preparations of *Vitis vinifera* as a single ingredient are considered for the monograph, while the studies performed with combinations are not discussed in this report.

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

The plant originates from North Africa, South Africa or South West Europe. *Vitis vinifera* L., Grape vine, is a perennial, defoliating limber with a wooden often twisted stem which can reach a length of 30 meters, but it is usually cut back to 1-3 meters. The shrub develops climbing branches forking to twigs from where the long-stemmed, alternating arranged leaves protrude. The vine leaf is heart-shaped, thin, long-stemmed, cordate with palmate venation and with 5-7 dentate lobes, divided by more or less deep and open sinuses. It can be up to 15 cm long and 12 cm across at its widest point, its colour is a uniform dark red. The upper surface is glabrous, but the lower surface may be pubescent. The venation is prominent on the lower surface. At the lower tendrils the flower panicles with numerous yellow-greenish flowers are formed. The fruits, arranged in large and long clusters are soft and pulpy berries with yellow-green, reddish or purplish dark-blue skin [Pharm. Franc. X., 1996].

Vitis vinifera belongs to the *Vitaceae* family. Several subspecies and varieties are distinguished among which is the subspecies *sylvestris* (Gmelin) Berger, recognised as the spontaneous form of *V. vinifera* L., the subspecies *caucasica* Vavilov, occurring in both wild and cultivated form. It is supposed that from these two, the cultivated form *Vitis vinifera* ssp. *sativa* DC has been grown [Bombardelli & Morazzoni 1995, Bombardelli et al. 1997].

- Herbal substance

The herbal substance consists of the dried leaves of the black to pulp-red grape and has a faintly perceptible odour. The herbal drug is harvested by hand in the autumn following the grape harvest. Drying takes place under natural conditions in accordance with local climatic conditions.

Latin Name: *Vitis viniferae folium* (*Vitis vinifera* L., var. *tinctoria*, *Vitaceae*) "Vitis vinifera" or "Vitis folium";

Common Names: vine leaves or vineleaves or vine leaf or vineleaf or grape leaf (English);

Feuilles de vigne (French), Rebenblätter, Weinlaub (German), Fogli della vite (Italian), Hogas de la vid (Spanish), Folhas da videira (Portuguese), Wijnstok bladeren (Dutch), liść winorośli właściwej (Polish), Φύλλο Αμπέλου (Greek).

The crude herbal substance complies with the monograph "Vigne Rouge" French Pharmacopoeia [Pharm. Franc. X., 1996]. The powder is reddish-brown. Examined under the microscope, the powdered red vine leaf shows the following characteristics: more or less dense, unicellular, long, tapering, covering trichomes, thick-walled, with bulbous or truncated base and a lumen divided into loculi; numerous raphides of calcium oxalate are contained in these cells or scattered about; fragments

Darné & Glories 1988]. From a pharmacological point of view, the polyphenols, for example flavonoids, are the most important substance group [Bruneton 1999; 2002; Raynaud, 2005; Diaz Lanza et al. 1995].

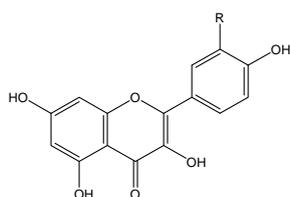
Analytically the following chemical compounds have been determined in the leaves of *Vitis vinifera*:

- *Flavonoids* (up to 3.5% for red vine leaf, the content is higher in green leaves 4-5%): including kampferol-3-O-glucosides, quercetin-3-O-glucosides
- *Tannins*: procyanidolic oligomers (proanthocyanidins, about 4%) including constituents monomers of catechin epicatechin
- *Stilbenes*: resveratrol and viniferins [Chung et al. 2003]
- *Fruit acids*: including tartaric acid, malic acid, succinic acid, citric acid, oxalic acid
- *Phenylacrylic acid derivatives*: p-coumaroyl acid, caffeoyl acid, feruloylsuccinic acid

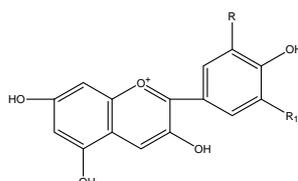
In a recent comparative study of 135 samples of grapevine leaves of different origin, the flavonol, anthocyanin and polyphenol contents have been determined. Total flavonol content was found to be between 0.6% and 3.5%, anthocyanin content between 0.2% and 1.45% and polyphenol content between 4.6% and 18.9%. HPLC methods used for determining anthocyanins and flavonols in grapevine leaves were validated and findings were compared to results produced by assays described in the French Pharmacopoeia. Whereas the correlation between conventional photometric and HPLC methods was satisfactory for anthocyanins, the correlation between the pharmacopoeia assay for total polyphenols and the HPLC analysis for flavonols was poor. As flavonol compounds are considered relevant for the vasoprotective effect of grapevine leaves, their content in starting material used in the production of herbal medicines needs to be quantified [Schneider et al. 2008].

Overview on main polyphenolic compounds in Grape Vine

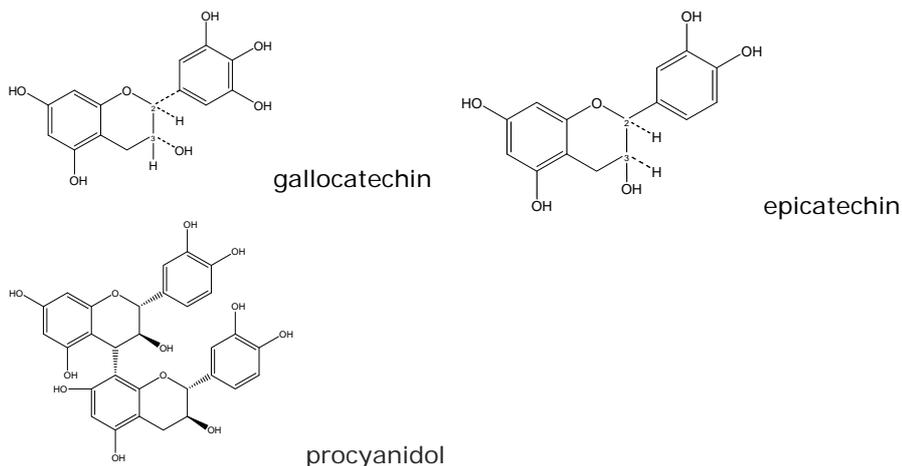
Flavonoids	<u>Flavones</u> (Quercetin Kaempferol), <u>Flavanes</u>
Anthocyanins	Leucoanthocyanidins, Anthocyanidins -responsible for the blue and red coloring of leaves, flowers and fruits -The concentration of anthocyanins in the red colored leaf is high.
Catechins	<ul style="list-style-type: none"> • Grapevine leaf is rich in catechins • Concentration of catechins is dependent on: ->the leaf's position on the plant the phase of development of the leaf • In autumn catechin, gallo catechin, epicatechingallat are present in the leaf.
Stilbenes	Resveratrol, <i>trans</i> -Resveratrol belong to the stilbenes (phenolic sub group) is a phytoalexin= stress-induced plant metabolite resveratrol can be found only in stressed leaves stress factors : i.e. fungal infection, UV-irritation, injury ->resveratrol is present in different forms depending on the stage of the plant's stress answer



flavonol



anthocyan



Primary pharmacodynamics

In Europe, the leaves of *Vitis vinifera* are documented in the literature of traditional medicine through several comprehensive reviews for their astringent and homeostatic properties where they are utilized in the treatment of diarrhoea, bleeding, haemorrhoids, varicose veins and other circulatory and venous diseases [Bombardelli 1997; Anonymous 2003; 2004; Petrini et al. 2003; Schneider, 2007i; 2007ii; Schneider et al. 2008; Schaefer & Red 2003; Volonté & Petrini 2004; Volonté et al. 2003; Weber, 2000].

Anti-inflammatory and anti-oedematous effect

In an *in vitro* study, venular endothelial cells were isolated from Wistar rats and cultivated on porous filters to confluent monolayers. These preparations respond to certain release products from simultaneously activated blood platelets and polymorphonuclear granulocytes (PMN) with a rise in hydraulic conductivity that, *in-situ*, would lead rapidly to local oedema, arteriolar constriction and venular thrombosis. In this model, selectively activated PMN alone induced only a modest increase in endothelial hydraulic conductivity that could be prevented by uric acid, an antioxidant. ASA prevented the activation of the blood cells. A standardized extract from grapevine leaves (AS 195), containing in particular the flavonoids quercetin-3-O-β-D-glucuronide and isoquercitrin (quercetin-3-O-β-D-glucoside), not only prevented the deleterious effect of the release products on the venular endothelial monolayers but, applied promptly to an endothelium damaged by prior exposure to these release products, resulted in the repair of the endothelium [Nees et al. 2003]. In another study, the scavenge by procyanidines (polyphenol oligomers from *Vitis vinifera* seeds CAS 85594-37-2) of reactive oxygen species (ROS) involved in the onset and the maintenance of microvascular injury has been studied in phosphatidylcholine liposomes (PCL) using two different models of free radical generation. In an iron-promoted (Fenton-driven) model, procyanidines had a remarkable, dose-dependent antilipoperoxidant activity (IC₅₀ = 2.5 μmol/l), more than one order of magnitude greater than that of the monomeric unit catechin (IC₅₀ = 50 μmol/l). In the second model, procyanidines were highly effective in preventing conjugated diene formation in both the induction (IC₅₀ = 0.1 μmol/l) and propagation (IC₅₀ = 0.05 μmol/l) phases. The scavenging effect of tocopherol was weaker with IC₅₀ of 1.5 and 1.25 μmol/l [Maffei Facino et al. 1994; Wollina et al. 2006].

The grapevine leaf extract is characterized by its content in flavonol glycosides and glucuronides, in particular quercetin-3-O-β-D-glucuronide, isoquercitrin (quercetin-3-O-β-glucoside), and kaempferol-3-O-glucoside. The most informative investigations were performed using confluent venular endothelial cells from animal and from human origin [Nees, 2003]. An attack by release products from simultaneously activated blood platelets (P) and polymorphonuclear granulocytes (PMN) leads to a breakdown of the venular endothelial barrier. Clinically this would result in formation of oedema and

constriction of microvessels. Red vine leaf RVLE extract was able to support the repair of the venular barrier after an attack by said release products. These effects could be demonstrated on cells of animal origin as well as on cells isolated from the human heart [Nees 2003i, 2003ii]. Quercetin-3-O- β -D-glucuronide that had been isolated from the extract and was shown to be the major metabolite being present in human plasma after ingestion of RVLE acted in the same way. The extent to which cellular gaps opened and allowed the supernatant to flow through could be measured as "hydraulic conductivity". 0.7 mg dry RVLE/ml incubation medium nearly prevented the opening of the barrier in the presence of activated PMN/P. Preincubation of the venular cell layer with 50 μ M quercetin-3-O- β -D-glucuronide for 7 days markedly reduced the hydraulic conductivity compared to untreated cells.

In a publication by [Jonadet et al. 1983], the authors describe studies conducted with anthocyanosides extracted from *Vitis vinifera* (a), *Vaccinium myrtillus* (b) and *Pinus maritimus* (c). The results obtained *in vitro* indicated that these compounds inhibit elastase, a proteolytic enzyme which plays a role in the deterioration of conjunctive tissue and elastic fibers and is involved in certain pathological vascular conditions. The IC50 values are 0.13 mg/ml for (a), 0.20 mg/ml for (b) and 0.31 for (c). Lineweaver-Burk curves revealed that the inhibition was not competitive. Results obtained *in vivo* show that the angioprotective activities of these compounds can be classified in decreasing order as follows: (a), (b) and (c).

Nees developed a measurable and reproducible *in vitro* experimental model to investigate the effect of substances capable of modifying the hydraulic conductivity of the endothelial barrier of the venules. In *in vitro* experiments, this extract has been shown to have a "sealing" effect on the endothelium of the venules and a protective action against fluid extravasation induced by incubation with chemical mediators of inflammation [Nees S et al. 2003i].

Red vine leaf extract (RVLE) prevented the deleterious effect of the release products of P and PMN on venular endothelial cells. In addition, the extract was able to support the repair of the venular barrier after an attack by said release products [Smith, 1999].

The anti-inflammatory activity of the oligomeric stilbene a-viniferin from *Vitis* has been also tested as well as its mode of action through inhibition of cyclooxygenase-2 and inducible nitric oxide synthase [Chung et al. 2003].

Hepatoprotective activity

The hepatoprotective effect of ethanolic extract and its four different fractions (CHCl₃, EtOAc, n-BuOH, and remaining water fraction) of *Vitis vinifera* L. leaves was investigated against carbon tetrachloride (CCl₄)-induced acute hepatotoxicity in rats. The ethanolic extract was found active at 125 mg/kg dose (per os). The ethanolic extract was fractionated through successive solvent-solvent extractions and the n-BuOH fraction in 83 mg/kg dose possessed remarkable antioxidant and hepatoprotective activities. Liver damage was assessed by using biochemical parameters (plasma and liver tissue MDA [malondialdehyde], transaminase enzyme levels in plasma [AST-aspartate transaminase, ALT-alanine transferase] and liver GSH [glutathione] levels). Additionally, the pathological changes in liver were evaluated by histopathological studies. Legalon 70[®] Protect was used AS standard natural originated drug [Orhan et al. 2007].

Antimicrobial activity

Ethanol extracts of *Vitis vinifera* (leaves, raw fruits, young branches; 2:1:1, v/v/v), were investigated for their antimicrobial activity against 14 pathogenic bacterial species and a yeast, *Candida albicans*, using the agar well diffusion method, 19 Turkish traditionally used medicinal plants. *Vitis* leaves showed broad-spectrum antimicrobial activity [Oskay & Sari 2007].

Antioxidative activity

The effect of grape leaf extract (GLEt) on antioxidant and lipid peroxidation states in liver and kidney alcohol induced toxicity has been evaluated. *In vitro* studies with DPPH^{*} and ABTS^{*} (cation radical) showed that GLEt possesses antioxidant activity. *In vivo* administration of ethanol (7.9 g/kg bw/day) for 45 days resulted an activity of liver marker enzymes (AST, ALT, ALP and GGT), lipid peroxidation markers (TBARS, lipid hydroperoxides) in liver and kidney with significantly lower activity of SOD, CAT, GPx, GST and non-enzymatic antioxidants (vitamin E, vitamin C and GSH) in liver and kidney AS compared with control rats. Administration of ethanol along with GLEt significantly decreased the activities of liver markers enzyme in serum towards near normal level. GLEt at a dose of 100 mg/kg was highly effective than 25 and 50 mg/kg body weight. In addition GLEt also significantly reduced the levels of lipid peroxidation and addition, significantly restored the enzymic and non-enzymatic antioxidants level in liver and kidney of alcohol administration rats. This observation was supplemented by histopathological examination in liver and kidney. These data suggest that GLEt exerts its protective effect by decreased the lipid peroxidation and improving antioxidants status, thus proving itself AS an effective antioxidant in alcohol induced oxidative damage in rats [Pari & Suresh 2008].

The antioxidative activity of the ethanolic extract of *Vitis vinifera* L. leaves was investigated. The ethanolic extract of *V. vinifera* leaves at 250 mg/kg dose was found to have the highest antioxidant activity [Sendogdu et al. 2006]. Comparable results have been published by [Kosar et al. 2007], from extracts from fresh, dried and brined leaves of *Vitis* [Monagas M et al. 2006].

Bronchodilatory activity

It has been investigated the effect of grape leaf hydroalcoholic extract on isolated rat tracheal contractions induced by KCl and acetylcholine. The trachea was removed from male adult Sprague-Dawley rat and placed in an organ bath containing Krebs-Henseleit solution and contractions were recorded isometrically. The results demonstrate that the grape leaf extract at 0.5, 1, 2, 4 and 8 mg/ml significantly reduces the tracheal contractions induced by KCl (60 mM) dose-dependently ($P < 0.0001$). Acetylcholine (55 μ M)-induced tracheal contractions were also attenuated at the same concentration of the extract ($P < 0.0001$). The grape leaf extract induced relaxation in the KCl-induced contraction in trachea was unaffected neither by nitric oxide (NO) synthase inhibitor (L-NAME, 100 μ M) nor by beta-adrenoceptor antagonist (propranolol 1 μ M). Our results suggest that the bronchodilatory effect of grape leaf extract is mediated via the voltage dependent calcium channels on the smooth muscle cells membrane. Furthermore, the beta-adrenergic and NO are not involved. The extract was prepared from dried grape leaves 50 g were wixed with 230 ml of ethanol 70% for 72 hours at room temperature and stirred four times a day. The mixture was filtered and the solvent evaporated. The obtained extract was 9.5 g [Gharib Naseri & Heidari, 2006].

Vasorelaxant effect on isolated rat aorta

The relaxant effect of *Vitis vinifera* leaf hydroalcoholic extract (VLHE) on isolated rat thoracic aorta contractions induced by phenylephrine and KCl, and the role of aorta endothelium on this action has been investigated. Rat aorta was removed and placed in an organ bath containing Krebs-Henseleit solution and aorta contractions were recorded isometrically. The results demonstrate that the VLHE at 0.125, 0.25, 0.5, 1 and 2 mg/ml reduces the endothelial intact aorta contracted by phenylephrine (1 μ M) significantly and dose-dependently. Endothelial denuded aorta showed the same relaxation but in much less extent. The IC_{50} of these two groups were 0.454 ± 0.08 and 1.73 ± 0.23 mg/ml respectively. VLHE also reduced the aorta contractions induced by KCl (80mM). The relaxatory effect of VHLE on KCl-induced contractions were less than those evoked by phenylephrine. Soluble guanylate cyclase inhibitor (methylene blue, 10 μ M) and nitric oxide synthase inhibitor (L-NAME, 100 μ M) reduced the VHLE-induced relaxation in the intact aorta significantly but, atropine (1 μ M) was unable to decrease this vasorelaxant effect. These results suggest that the most vasorelaxant effect of VHLE

on rat aorta is endothelium-dependent and also nitric oxide (NO) and cGMP are involved in this action [Gharib Naseri et al. 2004].

Spasmolytic effect

The effect of grape leaf hydroalcoholic extract (GLHE) on rat colon contractions induced by some spasmogens has been investigated. A piece of distal colon from male adult Wistar rats were dissected and mounted in an organ bath containing Tyrode solution and colon contractions recorded by an isotonic transducer under 1g resting tension. The GLHE (0.5- 4 mg/ml) reduced the contractions induced by KCl (60 mM), BaCl₂ (4 mM), acetylcholine (1 μM) dose-dependently (P<0.001). The spasmolytic effect of GLHE on ACh-induced contraction was unaffected by propranolol (1 μM), phentolamine (1 μM), L-NAME (300 μM), and naloxone (1 μM). In Ca²⁺-free but rich in KCl (120 mM) Tyrode solution, cumulative concentrations of CaCl₂ induced colon contractions which, were inhibited by the extract. Glibenclamide (3 μM) had no effect on the extract spasmolytic activity, but tetraethylammonium (5 mM) contracted the pre-relaxed colon induced by the extract. Results suggest that the grape leaf hydroalcoholic extract spasmolytic effect is due to the blockade of the voltage dependent calcium channels and activation of Ca²⁺-operated potassium channels [Gharib Naseri et al. 2006].

The effect of *Vitis vinifera* leaf hydroalcoholic extract (VLHE) on isolated rat tracheal contractions induced by KCl and acetylcholine has been also studied. The trachea was removed from male adult Sprague Dalwey rat and placed in an organ bath containing Krebs-Henseleit solution. The tracheal contractions were recorded isometrically under 1.5 g initial tension.

Results: The results demonstrate that the VLHE at 0.5, 1, 2, 4 and 8 mg/ml reduces the tracheal contractions induced by KCl (60 mM) significantly and dose-dependently (P<0.0001). Acetylcholine (55 μM)- induced tracheal contractions were also attenuated by the same extract doses significantly (P<0.0001). The VLHE-induced relaxation in the KCl-induced contraction in trachea was not affected neither by nitric oxide synthase inhibitor (L-NAME, 100 μM) or beta-adrenoceptor antagonist (propranolol 1 μM) and by muscarinic receptors antagonist (atropine 30 μM).

Conclusion: These results suggest that the relaxant effect of VLHE on rat trachea is evoked via voltage dependent calcium channel blockage and beta-adrenoceptors, NO and cholinergic receptors are not involved in this relaxant effect of VLHE [Gharib Naseri & Heidari, 2006].

Diuretic activity

Aqueous and alcoholic extracts of *Vitis vinifera* leaves were tested for diuretic activity in rats. The parameters studied on individual rat were body weight before and after test period, total urine volume, urine concentration of Na⁺, K⁺ and Cl⁻. In the present study alcoholic and aqueous extracts of *Vitis vinifera* leaves (100 mg/kg of body weight) showed increase in urine volume, cation and anion excretion. Furosemide was used as a reference diuretic [Shastry et al. 2002].

Secondary pharmacodynamics

Flavonoid-containing herbal preparations and isolated flavonoids have been reported to exhibit a wide range of biological effects, including antioxidant and enzyme-modulating actions and anti-allergic, anti-atherosclerotic, antithrombotic, antiviral, antibacterial, anti-inflammatory, antiproliferative, anticarcinogenic, antispasmodic and diuretic effects [Middleton et al. 1992, 1996; Hertog et al. 1996; Hollmann and Katan 1999; Pietta 2000].

In vitro tests

In the context with Chronic venous insufficiency (CVI) it would be of interest to evaluate whether grapevine leaf extract exhibits any effects on platelet aggregation. The effect of flavonoids on platelet aggregation was studied *in vitro* using platelet-rich plasma from four healthy male volunteers aged 24,