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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)	*Vitis vinifera* 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

of parenchyma containing twinned crystals of calcium oxalate; a few fragments of epidermis with polygonal cells and some reticulate venation.
Since the beginning of the last century in many studies the chemical constituents of the different parts of grape vine have been investigated. Fruit acids, tannins and pigments are the substances mainly responsible for taste, odour and colour [PDR for Herbal Medicines, 2004].

- Herbal preparation(s)

Comminuted herbal substance

Powdered herbal substance

Soft extract (DER 2.5-4:1); extraction solvent water

Dry extract (4-6:1; water)

See also market overview in 1.2

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Member State	**Regulatory Status**	**Comments**[1]
Member state	products, indications	Legal status
Austria	1) **360 mg-filmcoated tablet** 1 tablet contains: 360 mg dry extract (DER 4-6:1), extraction solvent water 2) **capsules** 1 capsule contains: 180 mg dry extract (DER 4-6:1), extraction solvent water 3) **oral drops** 1 ml contains 73.5 mg extract (corresponding 1.2 mg Aesculin) in ethanolic solution (the DER and the extraction solvent are not declared in the SPC at the moment) Since 1) December 2005 2) January 2000 3) March 1993 1) 1 tablet daily (up to 2 tablets). Duration of use: max. 3 months 2) 2 capsules in the morning (up to 4 capsules). Duration of use: max. 3 months 3) 30 drops in the morning in fasting state **Indications** 1,2) chronic venous insufficiency grade I and II; swelling of feet and lower leg	WEU

[1] Not mandatory field

Member State	Regulatory Status	Comments[1]
	3) phlebitis, edemas in the legs (also during pregnancy), varicose veins	
Belgium	**Hard-capsules** 350 mg powder per capsule powder, corresponding to 8% total polyphenols and minimum 0.20% of anthocyanosides Since 2005 oral administration Adults: 1 to 2 capsules three times daily, during the meals with a large glass of water. Maximal dose is 6 capsules per day. Duration of use should be limited to 3 months. If symptoms do not improve during use, consult a doctor **Indications** used in case of subjective symptoms of venous insufficiency in legs, such as heavy legs, after exclusion of all serious pathologies such as phlebitis, thrombophlebitis and thrombosis	WEU
Bulgaria	Not known	
Cyprus	Not known	
Czech Republic	**(WEU)** Vitis viniferae folii extractum aquosum siccum (4–6:1), 180 mg – standardized to flavonoids content 3-7% **Pharmaceutical form** Capsule **Posology** 2 capsules corresponding to 360 mg of the extract in the morning, during first 3 weeks increase of the dose to 4 capsules is possible to reach faster relief since 2001?? **Indications** Prevention and therapy of symptoms of light or starting moderate forms of chronic venous insufficiency related to varicose veins such as swelling of the calves, heavy legs, tingling	
Denmark	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market.	
Estonia	Not known	
Finland	Not known	
France	**(WEU)** Soft extract since 1970 **Pharmaceutical form** eye drops **Posology** 1 drop 2 to 8 times daily. 1 g of extract/100 ml **Indications** uninfectious conjonctival irritations **TU** **Preparations** Powder since 1982 dry aqueous extract since 1987, 1990 All for oral use, in adults **Posology**	

Member State	Regulatory Status	Comments[1]
	1) 1 hard capsule 3 times daily (5 if necessary) 350 mg of powdered drug/capsule 2) 1hard capsule 2 times daily 200 mg of extract/capsule 3) 1 hard capsule 1 to 3 times daily containing 169 mg of extract/capsule **Indications** 1); 2); 3) - Traditionally used in the symptomatic treatment of functional disorders of cutaneous capillary fragility, such as ecchymosis, petechias, etc. - Traditionally used:, in subjective signs of venous insufficiency, such as heavy legs - in haemorrhoidal symptoms combination herbal preparations with Mélilot (*Melilotus*) Marron d'inde (*Aesculus hippocastanum*) Hamamélis (Hamamelis)	
Germany	**TU** **Preparations**: soft extract (2.5-4:1), extraction solvent: water at least since 1976 **Pharmaceutical form** cream **Posology** for cutaneous use in adults 10 g cream contain 282 mg soft extract **Indications** Traditionally used to relieve symptoms of tired legs **Risks** (adverse drug effects, literature) Allergic reactions, itching and erythema, worsening of skin irritation. Contact allergy and/or hypersensitivity reactions **WEU** 1) soft extract (4-6:1), extraction solvent: water 2), 3) dry extract (4-6:1), extraction solvent: water In the market 1), 3) at least since 1976 2) 2005 1) oral liquid 2) film-coated tablet 3) capsule, hard all for oral use in adults **Posology** 1) 1 x daily 6 ml liquid if necessary 1 x daily 12 ml liquid 10 ml (= 11.013 g) liquid contain 0.6 g soft extract 2) 1 x daily 1-2 containing 360 mg dry extract each 3) 1 x daily 2 containing 180 mg dry extract each if necessary 1 x daily 4 **Indications** For symptomatic treatment of chronic venous insufficiency, which is characterised by swollen legs, a feeling	

Member State	Regulatory Status	Comments[1]
	of heaviness, pain, itching, cramps in the calves at night **Risks** Common gastrointestinal complaints, nausea. Hypersensitivity reactions of the skin (itching and erythema, urticaria) have been reported	
Greece	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market	
Hungary	1)180 mg capsules 1 capsule contains: 180 mg dry extract (DER 4-6:1), extraction solvent water (2008) 2) oral drops 81.4 mg Vitis viniferae rubrae folii extr. spissum(4-6:1), corresponding to 1.2 mg aescin/ml (1997) 3) Varixinal capsules 180 mg dry native extract of *Vitis vinifera* L., folium (DER: 4-6:1) extraction solvent: water (2009) 1) 2 capsules once a day 2) 30 drops in the morning in fasting state 3) 2 capsules once a day **Indications** 1) chronic venous insufficiency; at stage of C_2, C_3 and C_{4A} according to CEAP 2); 3) For alleviating the discomfort feeling (fatigability, tension of the legs) of the lower extremities occurring in the course of the mild venous circulation disturbance 1); 3) It is not recommended during pregnancy and lactation. Adverse effects: Gastrointestinal disorders (Common) stomach and intestinal discomfort, nausea Skin and subcutaneous tissue disorders: (Uncommon) rash, itching, hives	
Iceland	Not known	
Ireland	Not known	
Italy		Food supplements only.
Latvia	**Preparations** *Vitis viniferae* folii extractum aquosum siccum (4–6:1) 180 mg – standardized to flavonoids content 3-7% since 2004 **Pharmaceutical form** Capsule **Indications** Prevention and treatment of signs related to chronic venous insufficiency	
Lithuania	Not known	
Luxembourg	Not known	
Malta	Not known	
Netherlands	Not known	

Member State	Regulatory Status	Comments[1]
Norway	Not known	
Poland	Vitis viniferae extractum siccum (5-7:1), extraction solvent: water, capsule hard Product allowed onto the market according to previous polish pharmaceutical low (less than 10 years). **Posology** oral use: adults, 2 capsules once a day (in the morning) **Indications** support of mild symptoms of chronic venous insufficiency	
Portugal	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market	One authorised combination product.
Romania	Not known	
Slovakia	**Preparations** Vitis viniferae folii extractum aquosum siccum (4–6:1) 180 mg – standardized to flavonoids content 3-7% since 2005 **Pharmaceutical form** Capsule **Posology** 2 capsules (360 mg) once a day, in the morning time. Total daily dose can be increased up to 4 capsules once a day, in the morning time **Indications** 1) Prevention and treatment of signs related to chronic venous insufficiency, which is usually expressed in connection with varicoses and oedema in the lower parts of legs, fatigue, pins and needles and pain in legs **Risks (adverse drug effects)** Allergic reactions including generalized rash and urticaria. Nausea, gastric complaints	
Slovenia	Not known	
Spain	**Preparations** **(WEU)** Vitis viniferae folii extractum aquosum siccum (4–6:1), 180 mg – standardized to flavonoids content 3-7% **Posology** 2 capsules (360 mg) once a day, in the morning time. Since 2001 **Indications** Prevention and treatment of signs related to chronic venous insufficiency, which is usually expressed in connection with varicoses and oedema in the lower parts of legs, fatigue, pins and needles and pain in legs **(TU)** Powdered herb. substance 270 mg/caps	
Sweden	No authorized herbal medicinal products containing *Vitis vinifera* as a single drug preparation are on the market	
United Kingdom	Not known	

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	(4-6.1, water extr tab, 2005, 180 mg caps, 2003, drops 73.5 mg/ ml, corresp. 1.2 (aesculin), 1993
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	WEU Caps 350 mg 1- 6/d
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Czech Republic	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Ext aq (4-6:1) standardized 3-7% flavonoids. Caps 180 mg, 2001
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
France	☒ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	WEU, eye drops Aqueous soft extract (6:1) since 1970 TU, powder(1989) or water extr. for oral use (1987)
Germany	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	dry water extr. (4-6:1), 180mg 1976, (360 mg) 2005 oral liquid, 10 ml contain 0.6 g soft extr. **TU** soft water extr. (2.5-4:1), 1976, cream, 10 g contain 282 mg soft extr.
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Hungary	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	(4-6.1, water extr tab, 2005, 180 mg caps, 2008 & 2009, drops 81.4 mg/ ml, corresponding. 1.2 (aesculin), 1997
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known

Member State	Regulatory Status				Comments (not mandatory field)
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Food Suppl. No product registered
Latvia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	WEU Ext aq (4-6:1) standardized 3-7% flavonoids caps 180 mg, 2004
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Poland	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Slovak Republic	☒ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	WEU Ext aq (4-6:1) standardized 3-7% flavonoids. Caps 180 mg, 2005
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Spain	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	WEU Ext aq (4-6:1) standard. 3-7% flavon. caps 180 mg Powdered herb. substance 270 mg/caps
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product registered
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Red grapevine leaves, from *Vitis vinifera* L. [Fam. *Vitaceae*], are rich in flavonoids including anthocyanins, oligomeric proanthocyanidins (OPCs) etc.
The knowledge of the medicinal potential of grape vine *(Vitis vinifera*) can be traced back far in history:

- In Europe, the leaves of Vitis vinifera are documented in the literature of traditional medicine 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 [Anonymous 2004; 2005; 2006; Bombardelli & Morazzoni 1995].
- In Turkish folk medicine, vine leaves are known to have a diuretic effect, while the juice of leaves have been used as an eye bath [Kosar et al. 2007].
- Native North American indigenous peoples used the leaf tea of related fox grape (*Vitis labrusca* L.) for treating diarrhoea, as well as for hepatitis, stomach aches and thrush and externally poulticed the wilted leaves for sore breasts, rheumatism, headaches and fevers. Other closely related Vitis species have been used similarly.
- In Indian Medicine: Grape is used for headache, dysuria, scabies, skin disease, gonorrhoea, haemorrhoids and vomiting [PDR for herbal Medicines 2004].

Since ancient times, beneficial effects on health have been ascribed to wine and vine leaves, as confirmed by numerous "recipes" reported in Egyptian papyri, the Sumerian tablets, the writings of Hippocrates of Cos (5th-4th century BC), Celsus (1st century AD), Galen (130-201 AD) and Paracelsus (1493-1541). The use of vine leaves has been documented. Retracing therapeutic literature from France showed the origin of using red leaves shortly after first incidence of the respective grape vine varieties called "teinturiers" [Schneider, 2007i]. The herbal substance and herbal preparations have a long history of use in folk medicine where French winegrowers collected the red vine leaves at the time of the grape harvest to make infusions and paste-like poultices from them. The infusions were filled into bottles and regularly ingested in small quantities. The paste from vine leaves was used for the treatment of swollen, painful legs. Nowadays, the extracts of grapevine leaves were developed into modern herbal medicaments mainly used against venous diseases and for its effects on microcirculation [Rabe et al. 2005; Volonté M, Petrini, 2004; Volonté et al. 2003]. The vine, fresh and brined or fermented are used as food (mixtures of rice and spices with or without meat are wrapped with vine leaves) and have been widely consumed as traditional foods (Dolmas) around the Mediterranean countries [Kosar et al. 2007]. They are also used for diarrhoea, vomiting and varicose treatment [Gharib Naseri & Heirari 2006].

There is evidence of the use of grapevine leaf outside France, in Italy in 1957, where the Biosedra preparation was also tested in vascular disorders in gynaecology, producing a positive effect on capillary fragility.

As early as 1960, a clinical study was published in Germany on the venous efficacy of a product, which contained a fluid extract of grapevine leaf, with a drug/extract ratio of 1:1. The content of anthocyanin was set at 600 µg/ml,which was subsequently changed to 1.2 mg bioflavonoids per ml (German *Rote Liste* 1974). In 1969, a company registered the preparation as a medicinal product, with the indications of varices, phlebitis, thrombophlebitis, calf cramps and leg oedema. The successor product, has been marketed since 1971. The capsules and tablets contain 180 mg and 360 mg of dried extract of grapevine leaves.

An overview of grapevine leaf components and its supposed pharmacological action as well as the polyphenol composition of *Vitis vinifera* can be found in the literature [Anonymous 2003; 2004; Petrini et al. 2003; Schneider, 2007i; 2007ii; Schneider et al. 2008; Schaefer & Red 2003].
Nonclinical *in vitro* and *in vivo* studies suggested protective effects of components from extracts of grapevine leaves on the venous system; e.g. procyanidines [Maffei et al. 1994; Constantini et al. 1999], and flavonoids [Nees et al. 2003]. For the extract preparation *(Extractum Vitis viniferae foliae aquosum siccum, 4-6:1)*, dried leaves of red varieties of *Vitis vinifera* L. (Vitis viniferae folium) which comply with the monograph described in Pharmacopée Français (10ème edition) for "vigne rouge" are

used. Thus, the herbal substance consists of the dried leaves of the black to pulp-red grape which finally undergo a specific production process resulting in a defined flavonol content in the dry extract preparation. While the whole dry extract preparation as such is considered as active agent, it is particularly characterized by its content of flavonol glycosides and glucuronides, i.e. quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-glucoside, and kaempferol-3-glucoside. These flavonoids are considered to contribute predominantly to pharmacological effects. The extract AS 195 contains a total of 4-7% of flavonol glycosides, quantified as quercetin-3-O-β-D-glucuronide.

Grapevine leaves and extracts thereof have been traditionally used for the treatment of symptoms associated with venous insufficiency for more than 70 years, in France. Extracts have been introduced in a number of European countries, e.g. Austria, Belgium, Czech Republic, Spain, Switzerland, Italy, and the United Kingdom. It is indicated for the prevention and treatment of Chronic venous insufficiency (CVI), associated with varicose veins including oedema of the lower leg, heavy or tired legs, sensation of tension, tingling and pain.

Based on the survey of products available in the Member states, the traditional use of red leaves preparations has been demonstrated especially in France, Germany, Spain and Austria, for at least 30 years.

Red vine leaf has been included in the French official list entitled "Avis aux fabricants concernant les demandes d'Autorisation de mise sur le marché" by virtue of which, the products containing it were subjected to a simplified registration procedure before the implementation of the Directive 2004/24/EC.
In France: "Vigne Rouge" (= red vine leaf) is regarded as one of the herbal drugs whose efficacy and safety has been proven by thorough literature studies and long-term traditional use. The Pharmacopèe Française X ed. includes the monographs of "Vigne rouge" and "Extrait de vigne rouge (sec)" and the traditional use of red vine leaves is discussed in "Rotes Weinlaub" [Schneider, 2007i; 2007ii].

Herbal medicinal products with red vine leaf have a long tradition in France. The official compendium "Médicaments à base de plantes", issued by the French department "Ministère des Affaires sociales et de la solidarité", released 1990, and comprised the long tradition of diverse herbals in France. There are listed many herbals that have been used in France for a long time, at least for about 15–20 years. [Les Cahiers de l'Agence 1998] state the use of red vine leaf for symptomatic relief of chronic venous disease ("No 015+016: " Traditionellement utilise dans le traitement symtomatique des troubles fonctionnels de la fragilité capillaire cutanée, tels que ecchymoses, pétéchies, etc."; No. 017+018: "Traditionnellement utilisée: - dans les manifestations subjectives de l'insuffisance veineuse telles que jambes lourdes; - dans la symptomatologie hémorroïdaire").
Different products prepared of water extracts of "Vigne Rouge" (5:1) have been available in oral forms, in France to treat blood vessels fragility in order to reduce the feeling of heavy legs and haemorrhoids related disorders. The posology range from 169 mg to 200 mg, two to three times a day, for four weeks:

Vigne rouge, capsules: 1 capsule contains 200 mg of grapevine leaves dry aqueous extract is used, a DER of about (4-6:1). The product is registered since 1987. The manufacturer recommends the use of 2 capsules per day (1 capsule mornings and one evening, with a glass of water). The product is traditionally used to reduce fine blood vessels fragility, in order to reduce the feeling of heavy legs and haemorrhoids related disorders.
OR
1 capsule contains 169 mg of grapevine leaves dry extract (3:1). The manufacturer recommends the use of up to 3 capsules per day. The product is traditionally used to reduce the feeling of heavy legs, haemorrhoids related disorder and fine blood vessels fragility.

An article from 'Deutsches Medizinisches Journal' published in 1960 by K. Güthenke, "Wesen und Behandlung der Bindegewebsschwäche, insbesondere der Veneninsuffizienz mit Weinblattextrakt" (Nature and Treatment of weak connective tissues, in particular venous insufficiency, ref.) describes the long traditional use of grapevine leaves for treatment of diseases of the leg veins, and demonstrates that vine leaf extracts have been already traditionally used before 1960, in Germany. Registrations for the active ingredient *Extractum vitis viniferae foliae aquosum siccum* exist since 1969. Since 1974 a solution for oral use has been available. 20 ml contained 1-2 g of extract from leaves of *Vitis vinifera* (1:4-5) and 0.02 g of aesculin. In 1999 aesculin was removed from the formulation. The recommended dosage is 2-3 x 30 drops per day. The product is traditionally used to treat diseases of the leg veins (f.e. varicose veins, chronic venous insufficiency), to reduce pains and feeling of heavy legs, night time cramps in the calf, itching and swollen legs. The use of this product can be proven for over 30 years.

In 1991 the galenical form of hard gelatine capsules was registered and marketed for the first time. One capsule contained a combination of 180 mg of *Extractum vitis viniferae foliae aquosum siccum* and 3 mg aesculin (a coumarine glucoside that naturally occurs in the horse chestnut *Aesculus hippocastanum*). This herbal medicinal product was indicated for the treatment of venous insufficiency, varices, haemorrhoids, heavy tired legs and feet, essential oedema and calf cramps. The daily posology was 2-3 capsules a day. In 1999, aesculin was removed from the product and the only active substance declared was the dry extract of grapevine leaves. The capsules contain 180 mg of *Extractum vitis viniferae* (extraction solvent: water, DER: 4–6:1), with a recommended use of 2-4 capsules per day.
A cream (ointment) -containing soft extract (4-6:1; water) in a cream base
(10 g cream contain 282 mg soft extract)- has been traditionally used since 1976 to relieve symptoms of tired legs applying it several times daily.

Conclusions on traditional use
Based on information obtained from Member states and data retrieved from handbooks it can be concluded that the following extracts and uses of grapevine leaf extracts fulfil the criteria for traditional use:

Herbal substance

Not applicable.

Herbal preparations:

- Comminuted dried leaves
- Powdered dried leaf
- Soft extract (2.5-4:1; water) in a cream base

Concerning the preparation: dry extract (3:1; water), the product has been on the market since 1990 (France) and does not comply with the requirements of Directive 2004/24/EC for THMP. There is no complete information on the manufacturing process and DER of this preparation, and it is not possible to evaluate the similarity with other dry extracts under WEU.

2.2. Information on traditional/current indications and specified substances/preparations

Indication:

Traditional herbal medicinal product:

a) To relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.

b) For symptomatic relief of itching and burning associated with haemorrhoids.

c) For symptomatic treatment of cutaneous capillary fragility.

The product is a traditional herbal medicinal product for use in the specified indication exclusively based upon long-standing use.

2.3. Specified strength / posology / route of administration / duration of use for relevant preparations and indications

Posology:

-Dried leaves in herbal teas, 40 g/l. One cup (10g/250 ml) 2-4 times/day [ESCOP 2009]
-Comminuted total powder: 270-350 mg, 3 times/day (before the meals), up to 2100 mg/day
-Soft extract (2.5-4:1; water), in a cream base, (10g cream contain 282 mg soft extract), a thin layer should be spread on the affected area 1-3 times/day

-Dry extract (DER 4-6:1; water), single dose: 360-720 mg, daily dose: 360-720 mg

3. Non-Clinical Data

Non-clinical *in vitro* and *in vivo* studies suggested protective effects of components from extracts of grapevine leaves on the venous system; e.g. procyanidines [Maffei et al. 1994; Constantini et al. 1999], and flavonoids [Nees et al. 2003].

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Constituents

Vine leaves contain a wide range of polyphenol flavonoids including flavon(ol)-glycosides and glucuronides, quercetin-3-O-beta-D-glucuronide (most abundant of flavonoids), isoquercitrin, anthocyanins, oligomeric proanthocyanidins [Hmamouchi et al. 1997], catechin, epicatechin monomers and dimmers and gallic acid. The phytoalexin trans-resveratrol, another polyphenolic substance belonging to the stilbene group, can also be found in grape vine [Langcake et al. 1979]. It has been referred in the international literature that $AlCl_3$ is a potent elicitor of resveratrol production in vine leaves [Andrian et al. 1996]. In vine leaves, also organic acids appear, mainly malic and oxalic acid but also tartaric acid. Citric, fumaric and succinic acid can be detected in the leaves only in traces. Compared to the grape berries, grape leaves are richer in the content of carotenoids and vitamin C. According to the French Pharmacopoeia, the dried leaves of grapevine should contain at least 4% of total polyphenols and 0.2% of anthocyanins [Pharm. Franc. X., 1996; Jonadet 1983; Lardos & Kreuter 2000; Laparra et al. 1989].

The leaves of the red varieties are very rich in tannins from the catechin group. The composition in tannins of leaves depends on the phase of development and on their position on the plant. In autumn, catechin, gallocatechin and epicatechingallate can be found in the leaves. From catechins and/or leukoanthocyanidines so called oligomeric proanthocyanidins, colourless substances, are formed. The greatest part of anthocyans consists of malvidin glucosides but also delphinidin, cyanidin and petunidin glucosides occur [Laparra et al. 1989]. The highest content of anthocyans can be detected in the red leaf especially in autumn, in the time between the vintage and the shedding of leaves [Raynaud, 2006,

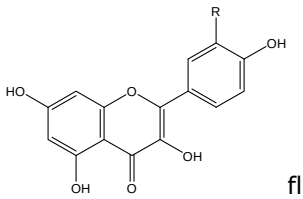
Darné & Glories 1988]. From a pharmacological point of view, the polyphenols, for example flavonoids, are the most important substance group [Bruneton 1999; 2002; Raynaud, 2005; Diaz Lanza et al. 1995].

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-3-0-glucosides, quercetin-3-O-glucosides
- *Tannins*: procyanidolic oligomers (proanthocyanidins, about 4%) including constituents monomers of cathechin 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

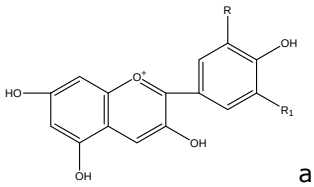
In a recent comparative study of 135 samples of grapevine leaves of different origin, the flavonol, anthocyanin and polyphenol contents have been determined. Total flavonol content was found to be between 0.6% and 3.5%, anthocyanin content between 0.2% and 1.45% and polyphenol content between 4.6% and 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

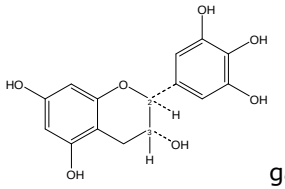
Flavonoids	Flavones (Quercetin Kaempferol), Flavanes
Anthocyans	Leucoanthocyanidins, Anthocyanidins -responsible for the blue and red coloring of leaves, flowers and fruits -The concentration of anthocyans in the red colored leaf is high.
Catechins	• Grapevine leaf is rich in cathechins • Concentration of cathechins is dependent on: ->the leaf's position on the plant the phase of development of the leaf • In autumn cathechin, gallocathechin, epicathechingallat are present in the leaf.
Stilbenes	Resveratrol, *trans*-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



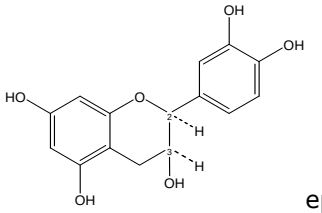
flavonol



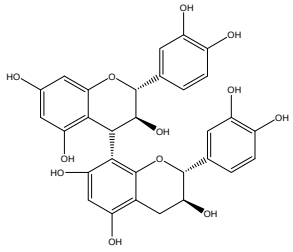
anthocyan



gallocatechin



epicatechin



procyanidol

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-ß-D-glucuronide and isoquercitrin (quercetin-3O-ß-D-glucoside), not only prevented the deleterious effect of the release products on the venular endothelial monolayers but, applied promptly to an endothehium damaged by prior exposure to these release products, resulted in the repair of the endothehium [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 af 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 from animal origin as well as on cells isolated from the human heart [Nees 2003i, 2003ii]. Quercetin-3-O-ß-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 µM quercetin-3-O-ß-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 though 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 ($CHCl_3$, EtOAc, n-BuOH, and remaining water fraction) of *Vitis vinifera* L. leaves was investigated against carbon tetrachloride (CCl_4)-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 µM)-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 µM) nor by beta-adrenoceptor antagonist (propranolol 1 µM). 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 mu 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 mu M) and nitric oxide synthase inhibitor (L-NAME, 100 mu M) reduced the VHLE-induced relaxation in the intact aorta significantly but, atropine (1 mu 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), $BaCl_2$ (4 mM), acetylcholine (1 mu M) dose-dependently ($P<0.001$). The spasmolytic effect of GLHE on ACh-induced contraction was unaffected by propranolol (1 mu M), phentolamine (1 mu M), L-NAME (300 mu M), and naloxone (1 mu M). In Ca^{2+}-free but rich in KCl (120 mM) Tyrode solution, cumulative concentrations of $CaCl_2$ induced colon contractions which, were inhibited by the extract. Glibenclamide (3 mu M) 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 Ca^{2+}-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 ($P<0.0001$). Acetylchline (55 mu M)- induced tracheal contractions were also attenuated by the same extract doses significantly ($P<0.0001$). The VHLE-induced relaxation in the KCl-induced contraction in trachea was not affected neither by nitric oxide synthase inhibitor (L-NAME, 100 mu M) or beta-adrenoceptor antagonist (propranolol 1 mu M) and by muscarinic receptors antagonist (atropine 30 mu M).

Conclusion: These results suggest that the relaxant effect of VHLE 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 VHLE [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,

29, 35 and 47 years. The flavonol glycoside quercetin 3-O-β-D-glucoside and various flavonoid aglycones including quercetin, apigenin and (+)-catechin were added to both platelet-rich plasma and washed platelets at concentrations of 0, 0.25, 2.5, 25, 250 and 2500 µmol/l. Indomethacin was used as a positive control. The results showed that the flavonoid aglycones inhibited platelet aggregation but that quercetin-3-O-β-D-glucoside did not significantly affect aggregation at any of the concentrations tested [Janssen et al. 1998].

In vivo tests
A randomised, placebo-controlled, multiple crossover study was conducted, in which 18 healthy volunteers (9 men and 9 women; mean age 25 ± 8 years; BMI 22 ± 1 kg/m^2) were given a preparation of onions equivalent to 114 ± 3 mg quercetin every day for 2 weeks in accordance with a fixed schedule. Blood samples were taken from the participants at defined intervals and the relationship between platelet aggregation and flavonoid concentration was plotted. The results showed that a daily dose of 114 mg quercetin did not have any significant effect on platelet aggregation. The mean plasma concentration of quercetin was 447 ± 117 ng/ml during the study period [Janssen et al. 1998].
Besides the flavonoids, grapevine leaves contain the following types of compound that are likely to be carried over to an aqueous extract [Bombardelli & Morrazoni 1995]:

- anthocyanins
- phenolic acids
- catechins and proanthocyanidins (flavanols)
- anorganic acids, sugars and mineral salts

The anthocyanins present in grapevine leaves are mainly glucosides of cyanidine, peonidine and malvidine [Boucheny A, Brum-Bousquet 1990]. Anthocyanins have been reported to possess antioxidant and vasoprotective properties. There are some doubts whether these effects measured in *in vitro* experiments or in animal studies using high doses are relevant for humans [Prior, 2004]. Absorption of anthocyanins appears to be low [Lapidot et al. 1998]. The amount of anthocyanins administered with 360 or 720 mg grapevine leaf extract is considered negligible in respect of the pharmacodynamic action of the substance.
The phenolic acids present in grapevine leaves are mainly derivatives of cinnamic acid, vanillic acid and caffeic acid [Boucheny A, Brum-Bousquet 1990]. These compounds are ubiquitous in vegetables and preparations of herbal origin and therefore not expected to contribute to the specific pharmacological effects of grapevine leaf extract.
The catechins found in grapevine leaves are mainly catechol and epicatechol [Boucheny A, Brum-Bousquet 1990]. Of the condensed catechins (proanthocyanidins) procyanidins B1 and B2 are the prevailing compounds, followed by procyanidins B3 and B4. Procyanidins have been reported to possess antioxidant and anti-oedematous activity [Bombardelli & Morrazoni 1995]. The study did not investigate the form in which the flavonoids were resent prior to administration. Onions are known to be rich in quercetin glucosides, especially quercetin-4´-glucoside and quercetin-3, 4´-O-diglucoside. After administration of fried onions quercetin glucuronides can be detected in plasma [Aziz et al. 1998; Graefe et al. 2001]. It can be supposed that quercetin glucuronides do not influence platelet aggregation *in vivo*.

Hypocholesterolemic effect

Oral administration of procyanidins from grape seed produced a hypocholesterolemic effect in a high-cholesterol animal feed model (rats). Specifically it prevented an increase in total and LDL plasma cholesterol and a decrease in HDL [Tebib, 1994].
In normal and hypercholesterolemic rabbit aortas, OPCs significantly lowered the amounts of cholesterol bound to aortic elastin compared to controls [Wegrowski, 1984].

Antiproliferative activity

Procyanidin-rich fractions from grape *Vitis vinifera* extract showing different mean degrees of polymerization, percentage of galloylation (percentage of gallate esters) and reactive oxygen species-scavenging capacity were tested on HT29 human colon cancer cells. It has been observed that the most efficient fractions in inhibiting cell proliferation, arresting the cell cycle in G_2 phase and inducing apoptosis were the grape fractions with the highest percentage of galloylation and mean degree of polymerization. Additionally, the antiproliferative effects of grape fractions were consistent with their oxygen radical-scavenging capacity and their ability to trigger DNA condensation-fragmentation [Lizarraga et al. 2007].

Antidiabetic activity

The acute and subacute antidiabetic activities of the ethanolic extract of *Vitis vinifera* L., leaves were investigated. The acute effect was studied on the normoglycaemic, glucose hyperglycaemic and streptozotocin-induced diabetic rats; and the subacute effect was studied on same diabetic rats for 15 days. The blood glucose levels were measured by using blood glucose measuring strips based on glucose-oxidase method. The ethanolic extract of *V. vinifera* leaves at 250 mg/kg dose was found to possess a high antidiabetic activity. Mainly condensed tannins and flavonoids were suggested to contribute in the activity [Sendogdu et al. 2006].
The acute and subacute (15 days) hypoglycaemic and antihyperglycaemic effect of the two different doses (250, 500 mg/kg) of the aqueous extract from the leaves of *Vitis vinifera* L. were evaluated in this study. The aqueous extract was further fractionated through successive solvent extractions and the acute effect of different doses of its subfractions, 25 mg/kg for ethylacetate fraction, 80 mg/kg for *n*-butanol fraction and 375 mg/kg for remaining aqueous fraction were investigated using normal, glucose-hyperglycaemic and streptozotocin-induced diabetic rats. Blood glucose levels were measured according to the glucose oxidase method. Tolbutamide was used as a reference drug at a dose of 100 mg/kg. The antioxidant activity of the test samples was studied in the liver, kidney, heart tissues of diabetic rats by measuring malondialdehyde (MDA) and glutathion (GSH) levels. All results were compared to the diabetic control groups. The results showed that the EtOAc fraction was rich in polyphenolics and possessed a significant antihyperglycaemic and antioxidant activity equipotent with the reference hypoglycaemic agent (tolbutamide), when evaluated in diabetic rats [Orhan et al. 2006].

Other activities

The anti-herpes as well as the anti-mutagenic activities from the leaves and procyanidins from *Vitis vinifera* have been also assayed and published in the international literature [Girre et al. 1990; Liviero et al. 1993].

Pharmacodynamic interactions

Pharmacodynamic drug interactions of whole extracts or isolated constituents have not been reported. Various sources in the literature have suggested that the desired therapeutic effect of many flavonoids is usually obtained only after repeated oral intake of the respective preparations. This implies that the flavonoids and their metabolites accumulate in the body, and do not exert the pharmacodynamic effects seen in *in vitro* studies until a pharmacological threshold dose and relevant plasma levels has been reached. This hypothesis was confirmed by the results of a clinical study by [Kiesewetter et al. 2002]. A significant improvement in key CVI symptoms in the active treatment groups versus placebo could not be observed after 6 weeks, but became evident after 12 weeks of treatment. This could also explain why earlier studies reported a "small" or "undetectable" effect following single oral administration.

3.1.1. Assessor's overall conclusions on pharmacology

The aqueous and ethanolic extracts of *Vitis viniferae* folium have been used as adjunctive therapy for Chronic venous insufficiency (CVI), and have shown a potential benefit [Abascal & Yarnell 2007]. Based on available preclinical data, it can be concluded that the mechanism of action of orally administered extract in CVI is not well known.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Data on the pharmacokinetics of the whole extract or relevant components are not available.

Flavonoids are the substances which are regarded as being responsible for the pharmacological properties of grapevine leaf extract. In the plant, flavonoids are generally present in genuine form as glycosides. Grapevine leaf extract as it has been referred previously contains as major flavonoid components quercetin-3-O-glucuronide, isoquercitrin (quercetin-3-O-glucoside) and kaempferol-3-O-glucoside. The substances present in blood after ingestion of plant extracts containing polyphenols usually differ from the native compound, as they are metabolites resulting from digestive and hepatic activity. The examination of pharmacokinetics and metabolism of the major and active constituents of such plant extracts in humans is therefore strongly recommended [Manach et al. 2005].
Various sources in the literature have suggested that the desired therapeutic effect of many flavonoids is usually obtained only after repeated oral intake of the respective preparations. This implies that the flavonoids and their metabolites accumulate in the body and do not exert the pharmacodynamic effects seen in *in vitro* studies until a pharmacological threshold dose and relevant plasma levels has been reached. This hypothesis was confirmed by the results of a clinical study by [Kiesewetter et al. 2000]. A significant improvement in key CVI symptoms in the active treatment groups versus placebo could not be observed after 6 weeks, but became evident after 12 weeks of treatment.

The grapevine leaf extract represents a complex mixture of different classes of compounds, having each their own pharmacokinetic characteristics. A significant portion of the components of grapevine leaf extract belongs to the group of polyphenols, such as anthocyanins, proanthocyanidins, catechins and phenolic acids [Bombardelli & Morazzoni 1995; Boucheny & Brum-Bousquet 1990]. Recently, [Manach et al. 2005] presented a review of various bioavailability studies in humans concerning polyphenols. They came to the conclusion that anthocyanins and proanthocyanidins are absorbed to a very low extent only. As an exception, the major circulating forms of anthocyanins are the intact glycosides [Manach et al. 2004]. The polymeric nature and therefore high molecular weight of proanthocyanidins should limit their absorption through the gut barrier. Hydroxycinnamic acids such as caffeic acid are rapidly absorbed from the small intestine and are conjugated, in particular glucuronidated in the same way as flavonoids. However these compounds are naturally esterified in plant products (as chlorogenic acid) and this impairs their absorption. Only the colonic microflora possesses esterases which are capable of hydrolyzing these bindings. Consequently, the efficacy of absorption of phenolic acids is markedly reduced for their esterified forms compared with the free form [Manach et al. 2004].

3.2.1. Assessor's overall conclusions on pharmacokinetics

No information is available on pharmacokinetic interactions. No information is available on metabolites.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Single-dose toxicity

The toxicity of vine leaf aqueous extract (4-6:1) following a single oral dose was determined in SPF Wistar rats (3 males and 3 females per group). The doses used were 3000, 4000 and 10.000 mg/kg. There were no signs of toxicity and no mortalities over the 14-day observation period, and no pathological or histological changes were seen at autopsy.

The toxicity of vine leaf aqueous extract (4-6:1) following a single oral dose was also determined in CFI mice (3 males and 3 females per group). The doses used were 2000, 4000 and 10.000 mg/kg. There were no signs of toxicity and no mortalities over the 14-day observation period, and no pathological or histological changes were seen in autopsy [Rabe 2005; ESCOP, 2009].

Repeat-dose toxicity

The toxicity of vine leaf soft extract when given over a 90-day period was determined in SPF Mol-Wistar rats (12 males and 12 females per group) in a study which was subjected to QAU inspection and carried out in accordance with GLP standards. The animals were given oral doses of 25, 125 or 250 mg/kg/day, or a placebo. A satellite group given 250 mg/kg/day was subjected to follow-up observation. No mortalities occurred during the study period. Observations made during the study period on bodyweight, food intake etc. revealed no evidence of any systemic toxic effects due to the extract. The values found during haematological and clinical chemistry testing were within biological limits. The autopsy findings and the results of histological tests did not reveal any evidence of changes due to the extract. The doses of extract used were therefore judged to be non-toxic in the rat.

The maximum daily dose of extract is 720 mg, which represents a dose of 10.3 mg/kg for a person weighing 70 kg. This is about one-thousandth of the maximum dose of extract used in the single-dose toxicity studies. As the maximum dose used in these studies did not give rise to any signs of toxicity, it is extremely unlikely that any toxic effects will occur in man. Preparations containing vine leaf extract have been used in European Union countries for many years and there have been no reports of any harmful effects in man to date. On this basis, it is reasonable to assume that the products reviewed in this report will not pose any safety risk on therapeutic administration [ESCOP, 2009].

Genotoxicity

The mutagenic potential of vine leaf extract was assessed in a range of established *in vitro* and *in vivo* test models (Ames test, point mutation assay and chromosomal aberration assay). It was assessed in an Ames Salmonella/microsome plate assay using Salmonella typhimurium TA-100, TA-1535, TA-98, TA-1537 and TA-1538 with and without metabolic activation; S9 mix prepared from rat liver was used as the activation system. The assay was carried out in accordance with EU recommendations and OECD Guideline 471. Five concentrations of the vine leaf extract ranging from 8 to 5000 µg/plate were used, and positive and negative controls were also included. The results obtained did not provide any evidence to suggest that vine leaf extract has any mutagenic potential [ESCOP, 2009].

Mutagenic potential of grapevine leaf extract was also assessed in two independent point mutation assays carried out on the HGPRT locus of Chinese hamster V79 cells with and without metabolic activation; S9 mix prepared from rat liver was used as the activation system in each case. The assays were designed and conducted in accordance with the guidelines and recommendations on genotoxicity testing that were current at the time. On the basis of preliminary toxicity tests, a concentration range of 3.0 - 5000 µg/ml was selected for use in the assays. Positive EMS and DMBA controls as well as a

vehicle control were included. Neither of the assays provided any evidence to suggest that the extract used had any mutagenic potential in the concentration range tested [ESCOP, 2009].

A micronucleus assay was carried out on NMRI mice (5 males and 5 females per group) to establish the potential of vine leaf soft extract for causing chromosomal aberrations. The assay, which complied with the relevant EU and OECD guidelines, was carried out using oral doses of the extract of 1.0, 3.0 and 10.0 ml/kg. Positive controls (cyclophosphamide 40 mg/kg) and vehicle controls (sodium chloride 0.9%) were included. Under the conditions employed, there was no significant increase in micronuclei frequencies in polychromatic erythrocytes.

The methanolic and aqueous extracts from Greek varieties of *Vitis vinifera* demonstrated *in vitro* antimutagenic effects against mutagenicity induced by bleomycin and hydrogen peroxide in Salmonella typhimurium strain TA102 [Stagos et al. 2006].
Oligomericproanthocyanidins from grape seed demonstrated in vitro antimutagenic activity [Liviero et al. 1993].
In addition, among the pharmacologically active flavonoids (quercetin, quercetin 3-O-β-D-glucuronide and isoquercitrin) in the vine leaf extract used is known that quercetin has given positive results in the Ames test. However, the mutagenic potential of quercetin has not been confirmed in *in vivo* tests carried out in rats and mice [Hertog & Hollman 1996].

Carcinogenicity

No data is available. However, it was demonstrated that anthocyanin-rich extracts from bilberry (*Vaccinium myrtillus* L.), chokeberry (*Aronia meloncarpa* E.), and grape *(Vitis vinifera*) inhibited multiple biomarkers of colon cancer in rats [Lala et al. 2006].
There has also been no evidence in the literature so far to indicate that flavonoids as a whole have any carcinogenic properties. Indeed, a large number of *in vitro* studies have been carried out which suggest that flavonoids have anticarcinogenic effects. However, the results obtained *in vitro* have yet to be confirmed in *in vivo* test models. Depending on the type and quantity of food consumed, the human intake of flavonoids is between 100 and 1000 mg per day [Middleton 1996].

In toxicity and carcinogenicity studies carried out on quercetin, promoted by the FDA, four groups of 50 male and female F344 rats were given 40, 400 or 1900 mg/kg of quercetin, or a placebo, in feed over a period of two years. There was no evidence of carcinogenic activity in the female rats and only slight evidence of carcinogenic activity in male rats. The carcinogenic effects, which took the form of renal tubule hyperplasia and nephropathy, were observed only in the high-dose group. No evidence of chronic toxicity was found [NTP: Toxicology and carcinogenesis studies of quercetin (CAS No. 117-39-5) in F344 rats (feedstudies) 1992].

Reproduction toxicity

The maternal and embryofoetal toxicity of an orally administered aqueous preparation of vine leaf soft extract was investigated in pregnant Himalayan rabbits in a study which was carried out in accordance with GLP standards and subjected to QAU inspection. Four groups of animals (12 per group) were used. One group received a placebo whilst the other three groups were given doses of 300, 1000 or 3000 mg/kg/day on days 6-18 of gestation. All doses were administered in a volume of 10 ml/kg. No signs of maternal toxicity were seen during the observation period and there was no visible evidence of foetal retardation. Slight, non-significant skeletal retardations and variations were seen in the foetuses of animals receiving the highest dose (3000 mg/kg of the extract per day). These variations were within biological limits in all cases and did not occur in any of the other groups. There was no evidence of changes due to the extract in any of the other findings, and no teratogenic effects were seen. On the basis of the results obtained, the NOEL for the extract was defined as > 3 g.

In the reproduction study conducted with rabbits no evidence of any teratogenic effects was found. The recommended maximum dose is equivalent to a daily intake of 10.3 mg/kg of vine leaf extract. In the Leuschner study up to 3000 mg/kg/day of the extract, were applied without resulting in any teratogenic effect. The recommended maximum human dose of vine leaf extract is about 300 times lower than the highest dose used in the Leuschner study [Leuschner F, Mitterer KE, 1993]. Examination of the influence of *Extractum vitis viniferae* on the pregnant rabbit and the fetus by oral administration [LPT Report No. 7441/92. 1993; ESCOP, 2009].

Daily doses of 3000 mg/kg extract from grapevine leaves administered to female rabbits during organogenesis (6th to 18th day of pregnancy) did not exhibit teratogenic effects [Antistax® SPC, 2005].

3.3.1. Assessor's overall conclusions on toxicology

Several recent data on oral single-dose and repeat-dose toxicity, as well as on genotoxicity and reproduction toxicity of vine leaf have been reported.
In vitro and *in vivo* studies on the mutagenic potential of vine leaf extracts have not shown any evidence of mutagenic activity. Carcinogenic effects are not expected to occur, especially as vine leaf extract has been used in medicinal products for many years without any evidence of such effects being reported.

4. Clinical Data

4.1. Clinical Pharmacology

Pathophysiology of CVI

The term Chronic venous insufficiency (CVI) describes a clinical picture in which chronic venous diseases of diverse aetiology are manifested by similar or related symptoms and complaints. CVI is mostly caused by venous and capillary hypertension, which causes a persistent, chronic, venous stasis in the skin of the lower leg, manifested most characteristically upon postural challenge [Alguire & Mathes 1997]. The impairment of venous return that is responsible for the venous capillary hypertension can be caused by degenerative, dilating venous conditions of the superficial (primary varices), the transfascial (insufficiency of the perforating veins), or the deep system ("deep" varicosis, insufficiency of the main veins) [Jünger et al. 1994]. Chronically disturbed haemodynamics of deep or superficial veins due to obstructed venous segments or valvular incompetence lead usually to trophic changes in the inner ankle area of the lower limbs and disturbances in the microcirculation of the skin have been considered to be major contributors for skin changes associated with chronic venous hypervolaemia and venous hypertension. Cutaneous microangiopathy of clinical relevance such as enlarged, tortuous capillaries surrounded by micro-oedema contributes to the skin alterations in the lower limbs and determines the course of CVI [Fagrell 1995; Jünger et al. 1996]. CVI can be classified according to its haemodynamic, morphological, and clinical aspects. The clinical grading system, based on Widmer's classification, as well as other international classification systems, e.g. CEAP (Clinical Class, Etiology, Anatomy and Pathophysiology), [Widmer LK, 1981; Partsch H, 1994; Porter JM et al. 1995]. The most important therapeutic approaches include compression therapy, physiotherapy, surgical treatment and sclerotherapy.

Drug therapy

Drug therapy is a treatment option in incipient and mild to moderate Chronic venous insufficiency (CVI) (Widmer grades I and II, CEAP Clinical classes 2, 3, or 4a).

Topical drug treatment

Antioedema drugs are mostly plant derived substances for which an antiexudative and antioedematous action and efficacy have been confirmed in experimental studies and clinical trials. Experimentally, most of them have a membrane stabilising action that leads to a decrease in capillary permeability. Some of these substances also have venotonic properties.
Long-term treatment with antioedema drugs can also be considered in primary and secondary CVI or if invasive measures are not applicable [Gallenkemper et al. 1998]. The majority of these products contain plant extracts or semisynthetic derivatives of plant-derived substances. The most commonly used are coumarins (α-benzopyrone derivatives); saponins/horse chestnut seeds extracts; flavonoids (γ-benzopyrone derivatives); rutin, diosmin, and hesperidin; anthocyanins and procyanidins.
Procyanidins, like the anthocyanins, are flavonoids that are not available as monosubstances. Procyanidins are present in relevant amounts in standardised extracts such as grapevine leaf extracts (*Vitis vinifera*) and are formed through condensation reactions of flavonols [Hostettmann K et al. 1994].
The complex syndrome of symptoms involved in CVI led to investigate the activity of vine leaf preparations on two distinct parameters affecting CVI. On the one hand the effects of the product on microcirculation, on the other on the typical objective and subjective symptoms of CVI such as the presence of oedema and the typical CVI complaints "tired, heavy, and swollen legs", or tension and pain in the legs.

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.1.1.1. Assessor's overall conclusions on pharmacodynamics

At present, the mechanism of action of vine leaf water extract in chronic venous insufficiency cannot be considered clarified.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

The vine leaf extract is a complex extract containing more than 200 different identified substances. The clinical effects of the grapevine leaf extract cannot be attributed to a specific individual constituent but can be ascribed with reasonable plausibility to the extract as a whole. It is therefore virtually impossible to perform classical pharmacokinetic studies with the complete product and pharmacokinetic studies of individual constituents such as flavonoids will only provide limited information with regard to clinical efficacy.

Flavonoids (quercetin glucuronide, kaempferol glucosides) are quantitatively important constituents of the whole extract. The systemic bioavailability of flavonoids is probably relatively low and variable. Orally administered flavonoids are susceptible to pre-systemic metabolism by the intestinal flora. It is likely that the absorbed metabolites formed by the microflora in the gut contribute to the biological action of orally administered flavonoids. Systemic flavonoids are metabolised in the liver primarily by conjugation (methylation or sulfuronidation) and/or glucuronidation. The resulting metabolites are excreted either with the urine or with the bile into the intestine, where they undergo further metabolism. The pharmacokinetic input function of systemic flavonoids may well be further extended by this enterohepatic recycling process [Manach & Donovan 2004].

4.1.2.1. Assessor's overall conclusions on pharmacokinetics

Data on pharmacokinetics of vine leaf extract or relevant components are not available in humans. No reliable methods exist to determine simultaneously the plasma levels of all active ingredients present in the whole extract.

4.2. Clinical Efficacy

Several publications referring to human clinical studies with medicinal products containing *Vitis vinifera* were found in the literature.
Three of the published studies investigated the safety and efficacy of a dry extract of grapevine leaves (4-6:1) extraction solvent water (AS 195).
The safety and efficacy specific information of these studies is presented below.

The clinical information available for aqueous extracts of grape vine leaf investigates the activity on two distinct parameters affecting Chronic venous insufficiency (CVI). On the one hand the effects of the product on microcirculation, on the other on the typical objective and subjective symptoms of CVI such as the presence of oedema and the typical CVI complaints "tired, heavy, and swollen legs", or tension and pain in the legs.

Clinical parameters measured

In all clinical studies, changes in leg volume and/or calf and ankle circumference were included in the endpoints studied. All confirmatory studies planned to show efficacy on subjective symptoms of CVI used the lower leg volume determined by water displacement plethysmography as the primary efficacy endpoint. Other endpoints included the lower leg diameter (at calf height and at the ankle), visual analogue scales (VAS: 0-10 cm) for the main symptoms ("tired, heavy legs", "sensation of tension", "prickling sensation", and "pain in the legs"), and the global assessment of efficacy and tolerability by the patient and by the doctor according to a 4-point verbal rating scale (VRS: "good", "satisfactory", "not satisfactory", and "poor"). These measurements are consistent with the guidelines of the German Society for Phlebology.
In the double-blind, placebo-controlled cross-over study with two treatment sequences of 6 weeks each separated by a 4-week placebo washout period, microcirculation in the most affected CVI leg was measured by laser doppler fluxmetry (685 nm, penetration depth approximately 1 mm) and transcutaneous oxygen monitoring.
Patients were usually questioned about their subjective well-being. They were also instructed to spontaneously report any adverse events experienced. Laboratory tests (haematology, clinical chemistry, and urinalysis) and blood pressure and pulse measurements were carried out in most studies to record safety.

4.2.1. Dose response studies

From the results of [Kiesewetter et al. 2000], a single daily dose of 360 mg of grapevine leaf extract is as effective and well tolerated as a daily dose of 720 mg. On average, the higher dose regimen offers greater efficacy in terms both of the extent and duration of action, but is roughly equivalent with regard to subjective symptoms reduction. The lower dose, however, is considered to be sufficient in the majority of patients and has been chosen to reduce the intake of extract, thus most likely decreasing the likelihood of observing adverse events. Consequently, this is the dosage that has been approved in some marketing authorisations.
No pharmacokinetic or pharmacodynamic studies, however, were performed to support the posology and daily dose proposed.

4.2.2. Clinical studies (case studies and clinical trials)

Several publications referring to human clinical studies investigating the safety and efficacy of a dry extract of red *Vitis vinifera* leaves (4-6:1) extraction solvent water (AS 195) were found in the literature. Some others studies have been assessed in this AR, that originate from confidential in house files of Boehringer Ingelheim. The company allowed using those data for assessment and monograph/list entry establishment.

Double-blind, Placebo-controlled studies

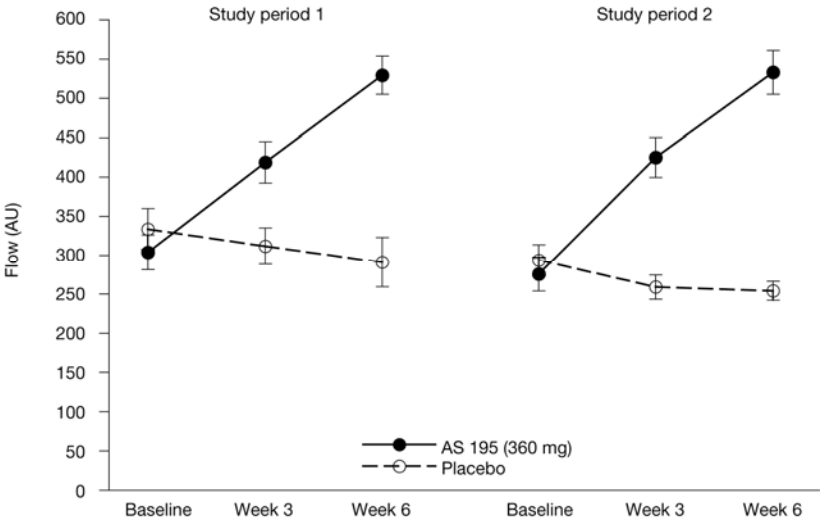
[Kiesewetter et al. 2000]: In a 12-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study conducted in accordance with the principles of Good Clinical Practice (GCP), the efficacy and safety of once daily doses of 360 and 720 mg RVLE (AS 195) were compared to placebo in male and female outpatients aged between 21 and 80 years old (with 52% ≥ 60 years) with body weights ranging from 46 to 120 kg (Broca index: median 7.3, range: -30 to +82%; this correspond to a BMI of approx. 25 kg/m^2). And stage I and incipient stage II Chronic Venous Insufficiency (CVI) with no major dystrophy of the skin. The majority (72%) of the patients were women. Compression therapy and the administration of diuretics were not permitted during the study period.
Patients were randomly assigned to a double-blind treatment with placebo, 360 mg RVLE or 720 mg RVLE once daily for 12 weeks, preceded and followed by a single-blind 2-week placebo treatment for baseline run-in and end-of-trial washout, respectively. Study criteria were evaluated at baseline, after 6 and 12 weeks of treatment and 2 weeks after discontinuation of treatment. Of the **260** patients enrolled and randomized, 219 completed the study in accordance with the protocol, 86 test subjects took a single daily dose of 360 mg AS 195, 84 took 720 mg AS 195/day and the remaining 87 test subjects received a placebo. During weeks 13 and 14 none of the participants received any further medication.

Subjectively, there was an improvement in CVI symptoms at 6 weeks with all treatments, but a further improvement at week 12 was seen only in the active treatment groups: at 12 weeks, the changes compared to baseline were significantly greater ($p < 0.001$) in both active treatment groups than in the placebo group. The study demonstrated that once daily doses of 360 and 720 mg RVLE were effective in the treatment of mild CVI, reducing significantly lower leg edema and improved CVI-related symptoms to a clinically relevant extent.

The treatments were well tolerated. All 260 patients who were enrolled and randomized were included in the safety analysis. This included three patients who withdrew from the study before visit 2 (i.e. before entering the treatment phase). 257 patients received at least one dose of the randomized study medication. Most patients completed the study up to 84 days of treatment. The mean duration of treatment for the 360-mg dose of AS 195 was 81.9 days (n = 86) whilst the mean duration of treatment for the 720-mg dose of AS 195 was 79.7 days (n = 84). Placebo was taken by 87 patients during the randomized treatment period (mean duration of treatment: 79.3 days) and by all patients during the 14-day run-in period (n = 260) and the 14-day follow-up period (n = 246). A total of 34 adverse events (AEs) were reported in 31/260 patients: 3 AEs in 3/260 subjects treated with placebo during the run-in phase; 3 AEs in 3/246 patients treated with placebo during the follow-up period; 10 AEs in 10/87 patients treated with 360 mg AS 195; 2 AEs in 2/85 patients treated with 720 mg AS 195; and 16 AEs in 13/88 patients treated with placebo. Most AEs were considered to be mild (n = 21) or moderate (n = 9). Four AEs were classified as severe, three in patients taking the placebo (phlebitis of the large saphenous vein; knee surgery; abdominal discomfort) and one in a patient taking 360 mg AS 195 (leg hematoma following minor trauma). Six AEs were rated as likely to be causally related to the study medication by the investigator; of these, two (mild constipation and mild hair thinning) were

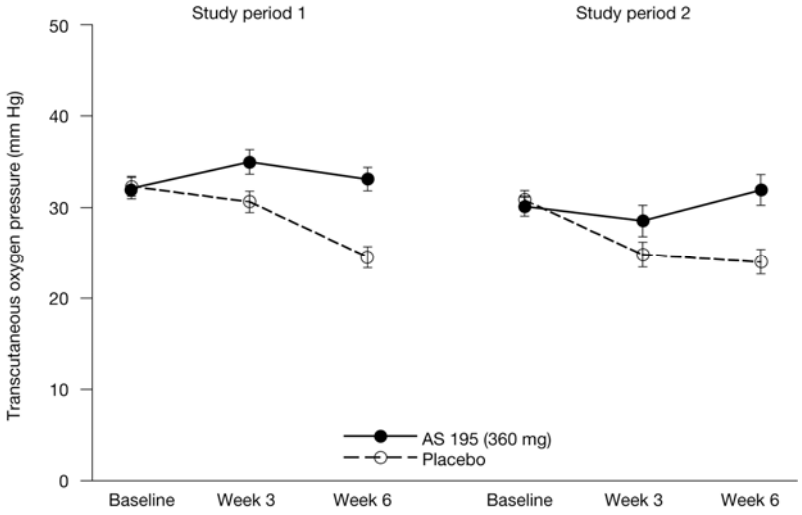
reported in patients treated with 360 mg AS 195, whilst the other four occurred in patients treated with placebo. Gastrointestinal disorders (abdominal discomfort, diarrhea, dyspepsia, dry mouth or retching) were the most frequently cited AEs (n = 11), followed by infections (n = 6), headache (n = 4) and disorders of the musculoskeletal system (n = 4). Two AEs which occurred during treatment with placebo required hospitalization and were labelled as „serious"; there was one incident of vaginal hemorrhage during the run-in phase and one incident of severe acute osteoarthritic complaints in the knee of the affected leg during the randomized treatment period. Three other patients were withdrawn following an AE: 1 patient was withdrawn during the placebo run-in period due to moderate abdominal discomfort; 2 patients were withdrawn from randomized treatment with the placebo, one because of phlebitis of the greater saphenous vein and one because of severe abdominal discomfort. The clinical laboratory data, blood pressure and pulse rate data and 12-lead ECG recordings did not indicate any significant mean or individual changes which were likely to have been due to the study medications. Overall tolerability was rated as follows for the 3 treatments: 360 mg AS 195: good 81%, satisfactory 19%; 720 mg AS 195: good 94%, satisfactory 6%; placebo: good 68%, satisfactory 30%, not satisfactory 2%. The study was conducted in multicentric settings, but most measurements were carried out by only two investigators, thus this trial can be considered basically a monocentric one

[Kalus et al. 2004]: The effect of AS 195 on cutaneous microvascular blood flow, transcutaneous oxygen pressure (tcpO2), and leg oedema was investigated in a randomised, double-blind, placebo-controlled, crossover study in male and female patients, aged ≥ 18 years with an average age of 66 years, with confirmed CVI stage I or II. In this efficacy study, **71** patients (70 during the second phase) were treated with either placebo or 360 mg AS 195 film coated tablets. The first group (n=36) received AS 195 360mg once daily during a first 6-week treatment period, followed by a 4-week placebo washout period and then placebo during the second 6week treatment period. The second group (n = 35) started with placebo and received AS 195 360mg after the placebo wash-out. After 6 weeks, patients in the AS 195 group had increased microvascular blood flow values and transcutaneous oxygen pressure (tcpO2). The active substance group showed a statistically significant increase of 241.8±18.7 Arbitrary Units (AU), while a reduction of 41.0±18.7 AU was recorded in the placebo group ($p<0.0001$).



Microcirculation, measured in arbitrary units (AU) (laser Doppler flow measurement at Doppler frequencies of 10.1–37.2 kHz, mean ± SEM, after 10 minutes of standing) (from [Kalus et al. 2004])

The $TcpO_2$ oxygen reading also rose significantly from baseline levels in the AS 195 group, by 1.35±0.97 mmHg, contrasting with a reduction in the placebo group of 7.27±0.97 mmHg.



Transcutaneous oxygen pressure (mean ± SEM, after 10 minutes of standing) (from [Kalus et al. 2004])

These changes in the measured parameters were associated with corresponding volume changes in the lower leg. After just three weeks of treatment, statistically significant differences were also observed in ankle and calf circumference. After six weeks, these circumferences had fallen by 0.39 and 0.54 cm respectively, in the active group, compared to increases in the placebo group of 0.29 and 0.14 cm respectively. The authors of the study concluded that the administration of AS 195 could have a positive effect on the course of CVI. The minimum number of days of exposure was 38 in the AS 195 and 24 in the placebo group, the maximum number 46 in the AS 195 and 45 in the placebo group. Thirteen (18.3%) subjects out of 71 experienced 16 adverse events (AEs) during the 16 week trial. Ten AEs (14.1%) were of mild, 4 (5.6%) of moderate and 1 (1.4%) of severe intensity. None of the AEs were assessed as related to the trial medication. One patient died from a cardiac arrest. He had been treated with placebo and never received AS 195 in this trial. All patients assessed the overall tolerability as good or satisfactory. Laboratory parameters did not change during the study [Kalus et al. 2004].

[Limoni C, 1996]: A four-week, multicentre, randomised, double-blind versus placebo, parallel-group safety trial to evaluate the tolerability profile of certain capsules (water extr. *Vitis vinifera* siccum 4-6:1) in male and female patients suffering from chronic venous insufficiency. The study population consisted of **105** patients, male and female, aged between 20 and 70 years with chronic venous insufficiency of grade I, II, and III according to Widmer. The patients received 3 capsules of that extract or placebo per day during 28 days. Seventy-two patients were randomised into the active treatment group and 33 into the placebo treatment group. In the absence of a strictly diagnosed CVI, patients with a subjective feeling of heavy and tired legs were admitted as well. Primary endpoints were the incidence of adverse events and the rating of the overall tolerability. Secondary endpoints were laboratory tests and physical examination. There were no differences in tolerability between the two treatment groups. The proportion of volunteers reporting adverse events was similar in both treatment groups: 40.2% in the active treatment group and 36.4% in the placebo group. The most frequent adverse events were mild gastrointestinal disorders, sleep disturbances, and tiredness. Laboratory values did not change in either treatment group during the trial period. The capsules were well tolerated by 84.9% of the volunteers. No severe adverse events were reported.

[Vix et al. 2007]: This 12-week, double-blind, randomised, placebo-controlled, multicentre trial was carried out to evaluate the efficacy and tolerability of film-coated tablets, extr. *Vitis vinifera* (***4-6:1 water***) 360 mg/day orally, in male and female patients suffering from chronic venous insufficiency. The design and methodology used closely reflected those used in BI trial by Schaefer 2004).
The time course of the change from baseline in limb volume was similar for both treatment groups with a more marked improvement of leg oedema over time in patients treated with these tablets. After 84 days of treatment, limb volume was reduced by -21.38 (±11.30) g of displaced water in the verum group. The adjusted mean reduction in limb volume in the placebo group was less pronounced with -10.71 (±11.56) g. However, the difference between treatment groups (-10.67 ±14.35 g) was not statistically significant.

For the secondary endpoint "change from baseline in calf circumference", the adjusted mean difference to placebo after 42 days of treatment with these tablets was -0.42±0.22 cm in the FAS (p=0.0596) vs. -0.31±0.24 cm on study day 84 (p=0.1851). In the Per-Protocol Set (PPS), the difference to placebo was -0.47 (±0.23) cm on study day 42, with a p-value just reaching significance at 0.0445. The evaluation of the four subjective symptoms of CVI, as assessed by VAS, yielded similar results: after 42 days of treatment, the adjusted mean differences between active treatment and placebo were -0.44 cm for "tired, heavy legs", -0.73 cm for "sensation of tension in the legs", and -0.48 cm for "pain in the legs" (vs. -0.38 cm, -0.04 cm, and 0.18 cm on study day 84). There was no difference between treatment groups regarding the symptom "tingling sensation in the legs". Global efficacy was rated as good or satisfactory by 70.9% of all patients treated with these tablets and 67.7% of all patients allocated to placebo whereas the investigators' ratings were 69.0% and 62.6%, respectively. The assessment of quality of life by the Tuebingen QoL questionnaire resulted in a better mean score for verum group compared with placebo regarding the subscale "leg complaints", the subscale most specifically related to CVI. The differences, however, were not statistically significant.

[Schaefer & Petrini 2004]: This placebo controlled, randomised, parallel group study was carried out in 2004. **247** CVI patients (CEAP-Scores 3 or 4a) were entered in the study: 121 were treated with the grapevine leaf extract AS 195 360 mg as a single tablet and 126 with placebo for 12 weeks. For the primary endpoint, the difference related to placebo, adjusted for centre effect and baseline values, in changes of displaced water from baseline until Day 84 was -10.42 cm (SE: 11.84; 95% CI: -33.76, 12.92). The difference in changes between the two treatment groups was not statistically significant. Subjective CVI symptoms diminished in both treatment arms similarly during the first three weeks. A statistically significant difference between the two treatment groups in favour of verum, was found for the symptoms "sensation of tension in the legs" at Day 42 (p=0.0211, ANCOVA) and "pain in the legs " at Day 84 (p=0.0222, ANCOVA). At all other time points no statistically significant difference was detected between the two treatment regimens for the symptoms "tired heavy legs" as well as "tingling sensation" in the legs. After 84 days of treatment, the limb volume as measured by the water displacement method was statistically not significantly more reduced in subjects allocated to verum than in those randomised to placebo. Careful inspection of the results showed that the unexpected high intra- as well as inter-individual variability of limb volume determinations contributed significantly to the outcome of the study. The variability between 2 or 3 replicates of the limb volume determination, for example, exceeded 20 g of displaced water in more than 20% of patients (range 0% to 44.1%) in 8 out of 23 study sites (35%). This study was negative in terms of a primary endpoint, although there was evidence of symptomatic benefit from the active treatment.

[Schaefer E & Petrini LE, 2003]: A 17 week, randomised, double-blind, placebo controlled cross-over trial to evaluate the efficacy of water extr. *Vitis vinifera* ***4-6:1*** film coated tablets (AS 195) given as 2 capsules of 180 mg of AS 195 daily 360 mg/day p.o., in **179** male and female Japanese patients with swellings of calf and ankle due to disorders of venous reflux belonging to Class 1 according to Porter's classification, in improving microcirculation of the skin in the leg of male and female patients

suffering from chronic venous insufficiency The microcirculation study has demonstrated that objective signs associated with CVI (microvascular blood flow, $tcpO_2$, ankle circumference and calf circumference) can be improved significantly after just 3 weeks of oral treatment with a single daily dose of 360mg of the grapevine leaf extract AS 195. These results build on those from Harrison trial (1998) showed that AS 195 reduces lower leg oedema and calf circumference in patients suffering from CVI treated once daily for 12 weeks. Out of the 179 evaluable subjects 38.5% experienced a marked and 42.5% a moderate global improvement of CVI symptoms. Improvement was rated as "moderate" by 59.8% of the patients with regards to heaviness/tiredness, 69.9% with regards to tension, 79.2% to tingling, 74.0% to pain, and 74.4% to itching. Circumference of calf (mean±SD) was 33.5±2.99 cm prior to treatment and 33.0±2.94 cm at 12 weeks, of the ankle (mean±SD) 22.3±1.84 cm prior to treatment and 21.9±1.82 cm at 12 weeks after treatment ($p<0.05$). These differences were not statistically significantly different from baseline.

Open-label, Observational studies

[Schaefer et al. 2003]: The 6-week, open, uncontrolled multicentre safety trial was conducted to specifically evaluate the tolerability and safety of AS195 film-coated tablets, 360 mg/day *per os*, in **65** male and female patients, aged 25 - 82 years, suffering from chronic venous insufficiency grade I or II (Widmer classification). The verum group received two film-coated tablets once daily for 42 days (360 mg/day). The primary objective of the study was to assess the tolerability and safety of AS 195 extr. *Vitis vinifera* siccum ***4-6:1***) film-coated tablets. The observational study was conducted in accordance with the requirements of the Swiss licensing authority (Swissmedic: application document) in a total of 11 study centres (general practitioners) between June and October 2002. Three visits (study enrolment, baseline examination at the start of the study, final examination at the end of the study, with a follow-up examination if necessary) were planned. The study was carried out in accordance with GCP. At the end of the study, all subjective symptoms of CVI (tired, heavy legs, sensation of tension in the legs, tingling sensations in the legs, pain in the legs) were statistically significantly improved. The global assessment of efficacy by the patients and by the investigators was rated as good or satisfactory in most of the patients. The primary variable was the number and intensity of adverse events (AEs), with particular emphasis placed on AEs regarded by the investigator to be treatment-related. Secondary safety variables included the global assessment of tolerability by both the patients and investigators as well as vital sign assessments. AS 195 film coated tablets were well tolerated during the whole study. Overall, six patients (9.2%) experienced a total of 7 AEs of mild or moderate intensity judged by the investigator as potentially causally related to study medication. They included gastrointestinal problems (4 patients), headache (1 patient), anorexia (1 patient), and erythematous rash (1 patient). Four patients of whom experienced a significant AE necessitated study discontinuation in three cases and dose reduction in one case. Most patients rated the global tolerability of AS 195 film-coated tablets as good (49 patients, 75.4%) or satisfactory (12 patients, 18.5%). Three patients (4.6%) rated tolerability as bad. One patient (1.5%) gave no rating. The global assessment of tolerability by the investigators was in good agreement with those of the patients. The investigators rated the tolerability as good in 50 patients (76.9%), as satisfactory in 10 patients (15.4%), as not satisfactory in one patient (1.5%), and as bad in two patients (3.1%). No relevant changes of vital signs (systolic and diastolic blood pressure, pulse rate) were measured. Patient´s compliance was rated as good [Schaefer et al. 2003].

Assessment The present open-label, multicentre observational study demonstrated that AS 195 film-coated tablets were effective and well tolerated in outpatients with chronic venous insufficiency grade I or II supervised in a setting of private practices. No change of the safety profile has been noted.

[Monsieur & Van Snick 2006]: A small, open observational clinical study was conducted in **39** patients suffering from Chronic Venous Insufficiency (CVI), grade I or II according to the Widmer

classification, (grade 2 to 4 of the international CEAP classification). Patients were treated with 180 mg of RVLE (AS 195) twice daily (360 mg in total) for six weeks. The parameters investigated were objective measurements of lower leg volume and the circumference of the leg as well as subjective criteria such as heaviness and pain in the leg. A clear and significant improvement of all parameters was observed after two weeks of treatment. This effect was still present and increased slightly after four and six weeks in all objective and subjective parameters tested in this study.

Assessment This study has shown a fast onset of action and an efficacy of the AS 195 extract in chronic venous insufficiency. Adverse events/side effects: not specified. In general, the treatments were well tolerated. Three patients prematurely stopped the trial for reasons not linked to the treatment or to the disease. No change of the safety profile.

Clinical studies with grapevine leaf extract (4-6:1, extract solvent: water) (AS 195)

Study ID	**Study Dates** **Persons**	**Design Control type Study objective**	**Study &, Ctrl Drugs Dose, Route, & Regimen**	**Subjects per Arm treated / completed**	**Duration**	**Gender M/F Median Age (Range)**	**Diagnosis Inclusion Criteria**	**Primary Endpoint**	**Outcome**
Kiese-wetter, 2000	April Sept-1998 **260**	randomized, double-blind, parallel, dose response Placebo Efficacy, tolerability, safety	AS 195 caps 180mg, *per os* 2-0-0 AS 195 capsules 360 mg, *per os* 2-0-0 Placebo	360 mg : 86/84, 720 mg :84/79 87/80	12 wks	24/62 56.2±12.4 years (mean±SD) 27/57 55.7±13.8 years (mean±SD) 20/67 59.2±11.5 years (mean±SD)	CVI Grade I, II according to Widmer	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmography	Positive
Schaefer & Petrini 2004	2004 **247**	randomized, double-blind, parallel Placebo Efficacy, tolerability	AS 195 tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0	360 mg : 121/117 126/123	12 wks, 2 wks single blind placebo	20/99 53.0 years (20–83 years) 15/111 53.0 years (23–79 years)	CVI CEAP-Scores 3 or 4a	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmography	Negative
Schaefer & Petrini 2003	2003 **179**	randomized, double-blind, controlled cross over trial Placebo Efficacy	AS 195 tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0	360 mg	17 weeks	No data	CVI Grade I, II according to Porter	Baseline adjusted changes of lower limb volume Changes in CVI symptoms	Negative
Vix et al. 2006	2006 **211**	randomized, double-blind, parallel Placebo Efficacy, tolerability	AS 195 tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0	360 mg : 103/98 99/95	12 wks	32/71 61.0 years (29–86 years) 26/73 61.0 years (30–87 years)	CVI CEAP Scores 3 or 4a	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmo-graphy	Negative
Kalus 2004	2002, completed **71**	randomized, double-blind, cross-over Placebo	AS 195 tablets 360 mg, *per os* 1-0-0	360 mg : 70/70	6 wks	16/55 65.2±7.7 years (mean±SD) (32–76)	CVI Grade I, II according to Widmer	resting flux, change from baseline measured by Laser Doppler fluxmetry	Increase of microvascular blood flow

Study ID	Study Dates Persons	Design Control type Study objective	Study &, Ctrl Drugs Dose, Route, & Regimen	Subjects per Arm treated / completed	Duration	Gender M/F Median Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint	Outcome
		Efficacy, tolerability	Placebo 1-0-0						
Schaefer et al. 2003	June 2001 Oct-2001 **65**	Open/ Observational Uncontrolled Tolerability, safety, Efficacy,	AS 195 tablets 180 mg, *per os* 2-0-0	360 mg : 65/59	6 wks	7/58 M: 65.9 years (mean) (56-82 years) F: 56.6 years (mean) (25–80 years)	CVI Grade I, II according to Widmer	Incidence and intensity of adverse events; Changes in CVI symptoms as secondary endpoint	Safety study Positive
Monsieur 2006	2006 **39**	Open Tolerability, safety, Efficacy	AS 195 tablets 180 mg, *per os* 2-0-0	360 mg	6 wks		CVI Grade I, II according to Widmer	Baseline adjusted changes of lower limb volume Changes in CVI symptoms	Positive
Limoni 1996	Dec-1996 May 1997 **105**	randomized, double-blind, parallel, dose response Placebo Multicentre Tolerability, Safety	AS 195 caps. 180 mg, *per os* 1-0-2 Placebo 1-0-2	540 mg : 72/64 33/27	4 wks	6/66 45.2 years (18.4–67.9 years) 4/29 36.7 years (25.6–70.6 years)	CVI Grade I, II. III according to Widmer	Safety Global improvement	Safety

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No information available.

4.3. Overall conclusions on clinical pharmacology and efficacy

The clinical efficacy of an extract from red vine leaves (RVLE; AS 195) was investigated in double blind, placebo-controlled studies [Kiesewetter et al. 2000; Kalus et al. 2004] and in open observational studies [Schaefer et al. 2003; Monsieur & Van Snick, 2006] during treatment of chronic venous insufficiency. In these studies, the RVLE was showed an acceptable clinical efficacy and tolerability, while there were some others with negative results [Schaefer & Petrini 2003; 2004; Vix et al. 2006]. Treatment with RVLE has been shown to lead to a reduction of leg volume, measured using both water plethysmography and calf and ankle circumference, and of the subjective symptoms investigated in the studies reviewed.

The extract AS 195 was consistently more effective than placebo in reducing leg oedema and improving symptoms of Chronic Venous Insufficiency (CVI) across most efficacy parameters evaluated, even though not always a statistically significant superiority could be shown.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

The safety profile of vine leaf extracts can be described as acceptable from all clinical studies in Chronic Venous Insufficiency (CVI) patients and from its use from products on the market. The safety results obtained from the clinical studies conducted so far show that the oral use of vine leaf extracts are well tolerated by most patients. No drug-related serious adverse events were reported during the clinical trials, and when reported that were mild and transient. Seven non-serious adverse events (constipation, dermatitis, headache, hair thinning, menometrorrhagia, urticaria) or moderate (erythematous rash) of mild intensity leading to patient withdrawals, were suspected to be related to the active trial medication.

5.2. Patient exposure

The controlled studies evaluated 1073 patients in total [Kiesewetter et al. 2000; Kalus et al. 2004; Schaefer & Petrini 2004; Vix 2006, Limoni 1996]. The open studies, evaluated by [Schaefer et al. 2003] and by [Monsieur and Van Snick, 2006] comprised 104 patients.

5.3. Adverse events

No health hazards or side effects are known in conjunction with the proper administration of designated therapeutic dosages. A reversible inhibition of intestinal enzyme activity (alkaline phosphatase, sucrose and dipeptidyl peptidase) was demonstrated in animal models [Tebib, 1994; PDR for Herbal Medicines, Thomson, 2004].

Brito et al. [2008]; Mur et al. [2006]: Vine pollen allergies have been reported with *Vitis vinifera* pollen and an extract thereof In Castilla-La Mancha, Spain, the area with the highest density of vineyards in the world. Two cases of allegic reactions to *Vitis vinifera* pollen and grape have been previously reported. The aim of a prospective study by Brito et al. [2008] was to determine the clinical relevance and biochemical characteristics of vine pollen in the Spanish province of Ciudad Real. The authors designed a prospective study of patients treated in the allergy units from Puertollano and Ciudad Real for respiratory symptoms of 4 months' duration in the year 2000. Skin prick tests with a standard aeroallergen battery and *V. vinifera* pollen extract were performed on all patients. The authors also performed conjunctival and bronchial provocation tests and serum specific IgE and sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting on the patients who tested positive for *Vitis vinifera* pollen.

The results of this prospective study show that *V. vinifera* pollen has a moderate clinical relevance from an allergic point of view, particularly affecting those subjects who are exposed to it during the working hours and those who perform leisure activities close to vine fields. During a period of four months, the authors performed skin prick tests to vine pollen in patients who attended the outpatient clinic with suspected respiratory allergy, finding sensitisation to *Vitis vinifera* pollen in 9 of the 200 patients seen (98 patients had allergy to other pollen). In conclusion, in areas with a high density of vineyards, vine pollen can reach midrange air concentrations and could be the cause of hay fever in those individuals with the highest level of exposure [Brito et al. 2008; Mur et al. 2006].

Assessment: In summary, there is no new safety specific information provided. No change of the safety profile is suggested.

Reports on immunoglobulin E (IgE)-mediated allergic reactions to grapes and wine are limited in the literature. Nevertheless, grapes are widely grown and consumed in Mediterranean countries. The

object of a prospective study by Kalogeromitros et al. [2005] was to present clinical features, *in vivo* and *in vitro* allergy testing, and human leukocyte antigen (HLA) serotyping in patients with recurring reactions to grapes and grape products. Eleven unrelated Greek patients, six men and five women (aged 16-44 years; mean, 26.9 years) were enrolled based on a documented history of IgE-mediated reactions to grapes, wine, or other grape products. Their evaluation included full history, reaction severity, clinical examination, skin-prick tests with food allergens and molds, serum IgE, specific IgEs to the same allergen battery, and HLA typing. Patients reported 35 grape-induced episodes of anaphylaxis ranging from moderate to severe. The authors described several cases of repeated IgE-mediated reactions to grapes, vine and other grape products. Similar clinical manifestations in different courses of grape allergen, almost identical prevalent co-sensitisations to other already known allergenic fruits, and the first determination of HLA antigens in grape-allergic patients are the core of this study.
The authors suggest that allergy to grapes may not be as uncommon as generally believed in the literature and should be considered as a possible offending cause in certain episodes of food-induced anaphylaxis probably in genetically at-risk individuals. However, allergic reactions to *Vitis vinifera* are known and reported in rare cases [Kalogeromitros et al. 2005].

Assessment: If hypersensitivity reactions are labelled correctly in the PIL and SPC, there is no change of the safety profile of medicinal products.

[Crowell et al. 2004]: Resveratrol-Associated Renal Toxicity. Resveratrol, (3, 5, 4'-trihydoxystilbene) a compound found in grapes, mulberries, and peanuts, has antimycotic, antiviral, and beneficial cardiovascular and cancer preventive activities. To evaluate the potential toxicity of resveratrol, rats were administered by gavage 0, 300, 1000, and 3000 mg trans-resveratrol per kilogram body weight per day for 4 weeks. Most of the adverse events occurred in the rats administered 3000 mg per kilogram body weight per day. These included increased clinical signs of toxicity; reduced final body weights and food consumption; elevated BUN (blood urea nitrogen), creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, and albumin; reduced hemoglobin, hematocrit, and red cell counts; and increased white cell counts. Increases in kidney weights and clinically significant renal lesions, including an increased incidence and severity of nephropathy, were observed. Diffuse epithelial hyperplasia in the bladder was considered, equivocal and of limited biological significance. No histological effects on the liver were observed, despite the clinical chemistry changes and increased liver weights in the females. Effects seen in the group administered 1000 mg resveratrol per kilogram body weight per day included reduced body weight gain (females only) and elevated white blood cell count (males only). Plasma resveratrol concentrations in blood collected 1 h after dose administration during week 4 were dose related but were relatively low given the high dosage levels; conjugates were not measured. Under the conditions of this study, the no observed adverse effect level was 300 mg resveratrol per kilogram body weight per day in rats (corresponding to 21 g in a 70 kg human) [Crowell et al. 2004].

Assessment: These results cannot be transferred to medical products containing Vitis vinifera or extracts thereof.

In summary, there is no safety specific information that is relevant for medical products. The reported adverse events / side effects were mild to moderate. The majority of the above mentioned events are considered to be related to underlying diseases, incidental concomitant disorders or other coincidences but not causally related to *Vitis vinifera*. From these published adverse events, no change of the safety profile of *Vitis vinifera* can be concluded. According to current knowledge, gastrointestinal disorders and hypersensitivity reactions should be labelled in the PIL and SPC.

The Periodic Safety Update Report for *Vitis vinifera* has been compiled within the joint BAH PSUR project. It summarizes safety data from 01/Nov/2003 to 01/Sep/2008 [BAH 2008].

In the report period no new safety relevant issues were found in the literature. There were no reports, clinical or experimental studies identified that give reason for a change in the assessment of the safety and benefit/risk ratio of *Vitis vinifera*.

5.4. Serious adverse events and deaths

The case of a 51 years old female patient is reported. She was hospitalized due to an acute icterus, which had developed over 15 days and was associated with asthenia, nausea and anorexia. Beyond the mucocutaneous icterus, the clinical examination did not show any abnormality, especially no chronic hepatopathy. The laboratory parameters suggested a cytolysis due to an ALAT value that was twelve times higher than normal, γ-GT and alcalic phosphatase plasma concentrations two times higher than normal and total bilirubine of 87 µmol/L. Normal findings in the abdominal ultrasonic examination lead to the diagnosis of an acute hepatitis. The patient has a history of euthyroidism. The patient did not consume alcohol and was in a good nutritional condition. The etiologic examination of this hepatitis was negative for infections, immunological and metabolic causes. A hepatic punction-biopsy, revealed a discrete cholangitis with focal ductal hyperplasia, a major cholestasis with numerous biliary thromboses and intrahepatocytic biliary pigments, a focal hepatocellular necrosis, a lymphohistiocytic infiltrate of weak intensity without significant fibrosis. Altogether this suggested an acute cholestatic hepatitis with either viral or toxic origin. The review of the medical questionnaire revealed sporadic intake of 1 g of acetaminophen and two repeated intakes in an interval of one week, when the first symptoms had appeared. Additionally the patient took *Vitis vinifera tinctoria* (extract of red wine leaves) and Fumitory (extract of *Fumaria officinalis*) daily for two months, as "summer cure" for the treatment of heavy legs and as a stimulant. She stopped the intake of these two products three weeks prior to hospitalization, when the first general symptoms appeared. The patient had already taken *Vitis vinifera tinctoria* at the same time one year before. Fumitory was used for the first time. According to the official method used at the pharmacovigilance centres in France, this case is classified as plausible with a chronological C2 and a semiotic S3 score of two products concomitantly used with paracetamol [Bonnet et al. 2007].

Assessor's comment

This case is classified as serious because of a significant medical reaction including hospitalisation.
A causal relationship with *Vitis vinifera* cannot be excluded (plausible temporal relationship). However, the used herbal products are insufficiently described; additionally relevant information on the dosage are lacking. Moreover, the concomitant use of acetaminophen must be considered. Acetaminophen is well known for its hepatotoxicity.

5.5. Laboratory findings

None reported.

5.6. Safety in special populations and situations

The product is not suitable for patients with known hypersensitivity against the herbal substance, the plant family, the herbal preparation or to the excipients of the final product.

5.7. Intrinsic (including elderly and children) /extrinsic factors

Grapevine leaf is not intended for use in children, while no restrictions are known for its use in elderly.

5.8. Drug interactions

No drug interactions have been reported.

5.9. Use in pregnancy and lactation

Grapevine leaf should not be used during pregnancy and lactation as there are not data available.

5.10. Overdose

No information.

5.11. Drug abuse

No information.

5.12. Withdrawal and rebound

No information.

5.13. Effects on ability to drive or operate machinery or impairment of mental ability

No information.

5.14. Overall conclusions on clinical safety

The safety profile of grapevine leaf extracts can be judged as good from clinical studies and from its long term use and market availability. The available literature, on pharmacological and toxicological studies, does not give rise to safety concerns. A special focus was set on the polyphenolic constituents of the extract (proanthocyanidins, flavonoids) that can be linked with the pharmacological effects. The proof of an acceptable safety is not only given by the period of time over which this extract has been used and by the quantitative aspects of their use but also by PSURs data and the safety data obtained during the clinical trials.
Overall, *Vitis vinifera*, leaves can be considered as safe in herbal medicinal products with a positive benefit/risk ratio.

6. Overall conclusions

Well-established use

The clinical efficacy and safety of the dry aqueous extract of grapevine leaves (4-6:1) has been demonstrated in appropriately designed clinical trials and is supported by extensive use of the product in the market since 1999.

The above referred extract showed an influence to the microcirculation and transcutaneous oxygen pressure at the predominantly affected perimalleolar area of the leg in Chronic venous insufficiency (CVI) patients who were treated for six weeks. Moreover, the assayed grapevine leaves extract has shown to lead to a reduction of the volume of oedema and to improvement of the typical subjective symptoms of CVI such as tired, heavy and swollen legs or pain and tension in the legs.

Three double-blind, placebo controlled trials investigated the change in leg volume as measured by water plethysmography as the primary endpoint, another also the changes in ankle and calf

circumference in the secondary endpoints investigated. In several of the clinical trials objective and subjective symptoms of CVI appeared to be effectively reduced, while there were some others giving negative results such as the ones of Schaefer & Petrini 2004 and Vix et al. 2006. The trials were performed according to ICH-GCP.

An additional placebo-controlled and open safety and tolerability trial provided supportive evidence for the efficacy.

This extract can therefore be regarded as an active substance with a well-established medicinal use.

The use of herbal medicinal products prepared with this extract is not recommended during pregnancy and lactation and should not be taken in children and adolescents under 18 years of age, due to the lack of adequate data.

The proposed indication is:

- Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose veins, a feeling of heaviness, pain, tiredness, itching, tension and cramps in the calves.

Traditional use

Other extracts and herbal preparations from grapevine leaves have been widely used. The safe use of the extract can be stated on the basis of the well-known, long-lasting and traditional use of preparations of grapevine leaves in the folk medicine and as registered medicaments.. Based on the evaluation and assessment of the present documentation of the clinical efficacy and safety, the herbal substance for use as herbal tea or in other oral dosage forms, and the aqueous extracts of grapevine leaf are acceptable as traditional herbal medicinal products.

Sufficient data are available to develop a Community monograph on the well established and traditional use of *Vitis vinifera* L., leaves. The indications are suitable for self-medication. The proposed indications are:

- Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.
- Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids.
- Traditional herbal medicinal product for symptomatic treatment of cutaneous capillary fragility.

The use of the traditional herbal medicinal products is not recommended during pregnancy and lactation and should not be taken in children and adolescents under 18 years of age.

The minimum required data on mutagenicity (Ames test) are available for herbal preparations of grapevine leaves. **The inclusion in the Community list of traditional herbal substances and preparations is recommended.**

Annex