EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

12 March 2013
EMA/HMPC/44208/2012
Committee on Herbal Medicinal Products (HMPC)

# Assessment report on *Sambucus nigra* L., fructus

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

Draft

| | |
|---|---|
| Herbal substance(s) (binomial scientific name of the plant, including plant part) | *Sambucus nigra* L., fructus |
| Herbal preparation(s) | Comminuted herbal substance |
| Pharmaceutical form(s) | Herbal substance or comminuted herbal substance as herbal tea for oral use. |
| Rapporteur | |
| Assessor(s) | |

This Assessment Report is published to support the release for public consultation of the draft public statement on *Sambucus nigra* L, fructus. It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation', will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft public statement.

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
**Telephone** +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
**E-mail** info@ema.europa.eu **Website** www.ema.europa.eu

# *Table of Contents*

# 1. Introduction

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

- Herbal substance(s)

The herbal substance consists of the dried, ripe berries of *Sambucus nigra* L. (elderberry). The wrinkled, more or less spherical dropes are dark violet-black and slightly glossy with a size around 0.5 cm. Usually, they contain three, somewhat elongated stones, each of which has a seed inside the hard endocarp. Fruit stalks are occasionally present. Elderberry belongs to the family Caprifoliaceae (Wichtl 2004). Elder berries taste sweet and sourish with a characteristic aroma (Bisset and Wichtl 2001, Wichtl 2004, Hiermann 2010).

The unripe berries contain toxic constituents such as cyanogenic glycosides and should be avoided in elderberry preparation (Pogorzelski 1982, Kunitz et al. 1984, Batz et al. 2005).

Fresh fruit is more or less spherical/round. Dried elderberry fruits are used as well as fresh fruit. However, only the dried fruits were considered for inclusion during the assessment work in support of the establishment of a draft Community herbal monograph.

- Herbal preparation(s)

Comminuted herbal substance

The available information on herbal preparations is described in section 2.2. 'Information on traditional/current indications and specified substances/preparations'.

- 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.

**Constituents**

The fruit of *S. nigra* L. contains several components that may contribute to pharmacological activity. Large amounts of anthocyanins are present in fresh fruits, the main constituents have been identified as cyanidin-3-glucoside (65.7% of total anthocyanins) and cyanidin-3-sambubioside (32.4% of total anthocyanins) (Brønnum-Hansen and Hansen 1983) in addition to small amounts of other types of anthocyanins, flavonols and flavonol ester (Roschek et al. 2009, Wu et al. 2004, Brønnum-Hansen and Hansen 1983). The dried seeds contain 0.1% hemagglutinin (lectins), identified as *S. nigra* agglutinin III (SNA-III, synonym SNA-IVf) (Peumans et al. 1991, Girbes et al. 1996). Another lectin, SNA-Vf (synonym nigrin f) has been found in fresh fruits (Van Damme et al. 1997, Citores et al. 1994, 1996 Girbes et al. 1996). Pogorzelski (1982) demonstrated the presence of cyanogenic glycosides in fruit but did not identify which ones; also it is not indicated whether the berries that were analysed were mature (Pogorzelski 1982). The fruits contain about 0.01% essential oil composed of 34 identified components (Wichtl 2004, Askar and Treptow 1985, Mikova et al. 1984, Davidek et al. 1982). Other ingredients are vitamins and minerals in small amounts and carbohydrates such as pectin and up to 7.5% glucose and fructose (Bisset and Wichtl 2001, Hiermann 2010, Askar and Treptow 1985).

**Table 1:** Constituents from the fruits of *S. nigra* L. (elderberries)

| Constituens | Amounts | References |
|---|---|---|
| ***Flavonoid*** | | |
| ***Anthocyanins*** | Total: **1-8 mg/g fresh fruits** | Brønnum-Hansen et al. 1985 |
| Cyanidin-3 glucoside (=chrysanthemin) | 65.7% of total anthocyanins in fresh fruits | Brønnum-Hansen and Hansen 1983 |
| | 739.8 mg/ 100g w/w in fresh fruits | Wu et al. 2004 |
| Cyanidin-3-sambubioside | 32.4% of total anthocyanins | Brønnum-Hansen and Hansen 1983 |
| | 545.9 mg/100g in fresh fruits | Wu et al. 2004 |
| Cyanidin -3,5-diglucoside | 0.8% w/w in fresh fruits | Brønnum-Hansen et al. 1985 |
| Cyanidin-3-sambubiosid-5-glucosid | 0.08% w/w in fresh fruits | Wu et al. 2004 |
| Cyanidin-3-rutinoside | 0.004% w/w in fresh fruits | Wu et al. 2004 |
| Pelargonidin-3-glucoside | 0.0018% w/w in fresh fruits | |
| Pelargonidin-3-sambubioside | Not available | |
| *Flavonols* | | Roschek et al. 2009 |
| 5,7,3,4- tetra-O-methylquercetin | Not available | |
| ***Favonol ester*** | Not available | |
| Dihydromyricetin-3-yl-3,4,5-trihydroxycyclohexanecarboxylate | | |
| ***Flavonol glycosids*** | Not available | |
| Isopquercitrin, Hyperoside, Rutin | | Hiermann 2010 |
| **Proteins** | | |
| ***Lectins found in seeds/fruits*** | | |
| SNA-IVf = identical to SNA-III (*S. nigra*-agglutinin-III) | 0.1% w/w in dry seeds | Peumans et al. 1991, Girbes et al. 1996 |
| | Not available | Mach et al. 1991 |
| SNA-Vf = identical to nigrin f | Not available | Van Damme et al. 1997, Citores 1996, Girbes et al. 1996 |
| Sam n 1 | Not available | Förster-Waldl 2003 |
| **Cyanogenic glycosides in seeds/fruits** | Not available | Pogorzelski 1982 |
| **Other Constituents** | | |
| ***Essential oil in fresh fruits - 0.01%*** | | |
| Phenylacetaldehyde | 35% of total essential oil | Davidek et al. 1982 |
| | 2.9% in fresh fruits | Mikova et al. 1984 |
| | 25.8% in fresh fruits | Askar and Treptow 1985 |
| 2-furaldehyde (furfural) | 18% of total essential oil | Davidek et al. 1982 |
| | 3.7% in fresh fruits | Mikova et al. 1984 |
| Butylacetate | Not available | Askar and Treptow 1985 |
| Isopentanol | | |
| 1-Hexanol | | |
| Acethyl furan | | |
| Benzaldehyd | | |
| Benzylalcohol | | |

| | | | |
|---|---|---|---|
| | α terpineol | | |
| | Ethylacetate | | |
| | 2-phenylethanol | 10.4 mg/kg in fresh fruits | Mikova et al. 1984 |
| | ***Fatty acids in fresh fruits*** | | |
| | Methyl Palmitooleale | 11.3 mg/kg | Mikova et al. 1984 |
| | Ethyl oleate | 28.5 mg/kg | |
| | Ethyl linolate | 77.5 mg/kg | |
| | Ethyl myristate | 4.3 mg/kg | |
| | Methyl palmitate | 5.3 mg/kg | |
| | Ethyl palmitate | 11.3 mg/kg | |
| | Ethyl palmitooleate | 7.7 mg/kg | |
| | Ethyl stearate | 8.1 mg/kg | |
| | Methyl linolate | 28.5 mg/kg | |
| | Methyl linolenate | 11.0 mg/kg | |
| | Ethyl linolenate | 50.0 mg/kg | |
| | Methyl oletate | 8.1 mg/kg | |
| | Linalool | 3.4 mg/kg | |
| | ***Organic acids*** | | |
| | Citric acid, malic acid, viburnic acid | Not available | *Souci 1989*, cited by Wichtl 2004 |
| | ***Vitamins and minerals in 100 g of fresh fruits*** | | |
| | Vitamin B 2 | 65 mg | *Souci 1989 * cited by Wichtl 2004, Askar and Treptow 1985, Hiermann 2010 |
| | Vitamin C | 18-26 mg | |
| | Folic acid | 17 mg | |
| | Biotin | 1.8 mg | |
| | β-carotene | 0.36 mg | |
| | Vitamin B6 | 0.25 mg | |
| | Pantothenic acid | 0.18 mg | |
| | Nicotinamide | 1.48 mg | |
| | Potassium | 288 mg-305 mg | |
| | Phosphorus | 49 mg-57 mg | |
| | ***Carbohydrates*** | | |
| | Glucose and fructose | 7.5% | *Souci 1989*, cited by Wichtl 2004, Bisset and Wichtl 2001 |
| | Pectin | 0.16% fresh fruits | Vulić et al. 2008 |

*Note* These references were not available.

**Note** Total amount of anthocyanins have been identified in different varieties of the elderberry and increasing with ripeness of the berries.

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

**Regulatory status overview**

| Member State | Regulatory Status | | | | Comments |
|---|---|---|---|---|---|
| Austria | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Belgium | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Bulgaria | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Cyprus | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Czech Republic | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | Only in combination |
| Denmark | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Estonia | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Finland | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| France | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Germany | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Greece | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Hungary | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Iceland | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Ireland | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Italy | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Latvia | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Liechtenstein | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Lithuania | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Luxemburg | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Malta | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| The Netherlands | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Norway | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Poland | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | Food supplements (as herbal tea and combinations) |
| Portugal | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Romania | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Slovak Republic | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |

| Member State | Regulatory Status | | | | Comments |
|---|---|---|---|---|---|
| Slovenia | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | |
| Spain | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Sweden | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| United Kingdom | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |
| Serbia | ☐ MA | ☐ TRAD | ☐ Other TRAD | ☐ Other Specify: | No products |

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.

Sambuci fructus is widely used across Europe in various food supplements, but no product containing Sambuci fructus as a single herbal substance/herbal preparation is authorised/registered according to the available information. Only combination products are reported to be on the market.

**Czech Republic**

In the Czech Republic, a fixed combination (herbal tea for oral use) has been on the market since 1972. This tea contains Fucus, Frangulae cortex, Sambuci nigrae flos, Sennae folium, Foeniculi fructus, Petroselini radix, Sambuci fructus, Betulae folium and Liquiritiae radix. This tea is used as a supplement in dieting regimens and in obesity complicated with constipation and fluids retention.

**Poland**

In Poland, two fixed combinations have been on the market for 21 years.

1) Herbal tea

The herbal tea in bags contains Frangulae cortex, Sennae folium, Carvi fructus, Sambuci fructus and Menthae piperitae folium.

Oral use: 0.9 – 1.4 g (168 – 252 mg of Sambuci fructus) once daily

The tea is used as a laxative in constipations.

The following adverse drug effects are listed: rarely itch, urticaria, rash in hypersensitive persons; rarely spastic pains abdominal cavity, watery stool; electrolyte and water balance disorders, potassium loss, Pseudomelanosis coli, albuminuria and hematuria in prolonged use.

2) Extract

Extractum compositum (1:2) ex: Helichrysi inflorescentia, Matricariae flore, Coriandri fructus, Sambuci fructus (24:15.5:7.5:3), extraction solvent: ethanol 60% (v/v) + Taraxaci intractum (1:1), extraction solvent: ethanol 96% (v/v).

This extract is traditionally used as a cholagogue in symptoms of dyspepsia.

Oral use: Adults: 5 ml (100 g of liquid contain 89 g of extract) up to 3 times daily

Children 14-18 years old: 2.5 ml up to 3 times daily

### *1.3. Search and assessment methodology*

The following electronic databases were searched on various dates from August 2011 to September 2012 with the search terms "*Sambucus nigra* fructus, Elderberry, and European elderberry".

**Results**

**PubMed:** (No case report of safety concern)
*Sambucus nigra* fructus: 38 references
*Elderberry:* 766 references
*European elderberry*: 18 references

**Toxline:**
*Sambucus nigra* fructus: 24
*Elderberry:* 204
*European elderberry*: 101

**Scifinder:** (Both chemical abstracts and Medline)
*Sambucus nigra* fructus: 13 references
*Elderberry*: 632 references
*European elderberry*: 5 references

**The Cochrane Library:**
*Sambucus nigra* fructus: No references are obtained
*Elderberry*: 9 references
*European elderberry*: 1 reference

Each database was searched from its start to the search dates. All languages were included.
Key text books were also searched for relevant studies. All references from the extracted papers were searched for citations not retrieved in the literature search.

# 2. Historical data on medicinal use

### *2.1. Information on period of medicinal use in the Community*

The traditional use of the elderberry goes back to ancient times. The traditional medicinal uses of elderberry against cold, as laxative, as diaphoretic and as a diuretic have been documented in scientific literature and several handbooks such as Madaus (1938), Grieve (1931), Bisset and Wichtl (2001) and Wichtl (2004).

Elder is native to Europe, west and central Asia and North Africa. The material of commerce is imported from Russia, Poland, Hungary, Portugal and Bulgaria (Wichtl 2004). Historically elder was a highly valued plant, because almost all the different parts of the plant were used, for various purposes, both as food and in folk medicine in Europe (Blochwich 1677, Vallès 2004). In Europe, the tradition has been to use both the fruits and flowers in folk medicine (Madaus 1938, Grieve 1931).

According to Madaus (1938), a decoction of dried elderberries was used as a laxative in Germany and a tea made from fresh elderberries has been used to the same purpose in Ukraine, Poland and Czechoslovakia since 1887. Elderberry syrup (Roob Sambuci) was also used as a laxative according to Grieve (1931) with reference to the British Pharmacopoeia 1788.

In his reference to the British Pharmacopoeia 1788, Grieve (1931) mentioned that elderberry syrup (Roob Sambuci) has traditionally been used against cold in parts of Europe, including England and Holland. According to Bisset and Wichtl, the drug is used as a diaphoretic in catarrhal complaints (Bisset and Wichtl 2001).

According to Madaus (1938) both fresh elderberries and elderberry juice were used as a diaphoretic in Europe and elderberry syrup (Roob Sambuci) was used to the same purpose according to information mentioned in Wichtl 2004 with reference to the Swiss Pharmacopoeia 1953.

A herbal tea made from dried elderberry fruit has also been used as a diuretic (Bisset and Wichtl 2001, Wichtl 2004).

Elderberry juice has also been used to treat sciatica, headache, dental pain, heart pain, nerve pain, especially in nerve pain and has been mentioned in handbooks such as Wichtl (2004) and Grieve (1931).

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

**Table 2:** Evidence regarding the traditional use and posology from handbooks

| Traditional use and therapeutic indication | **Posology** | **Reference** |
|---|---|---|
| Laxative | Roob Sambuci (elderberry syrup):<br>2.27 kg of fresh berries cooked with<br>453 g of sugar to the thickness of honey.<br>Taken in large doses. | British Pharmacopoeia 1788 cited by Grieve 1931 |
| Laxative | 1/2 teaspoon dried ripe berries in one cup of cold water, leave overnight and warm in the morning and drink. | Madaus 1938 |
| Laxative | Single dose: 2 g of the herbal substance as a decoction. | Czech National Norm 1958 replaced by Český farmaceutický kodex 1993 in which single dose, pharmaceutical form and area of therapeutic use are defined |
| Laxative | Herbal tea: 1 teaspoon/cup of the herbal substance as an infusion or decoction. | Karmazín et al. 1984 |
| Laxative | Roob Sambuci (elderberry syrup):<br>Fructus Sambuci recens 1000 T.<br>Aqua 200 T.<br>Saccharum q.s<br>Taken in large doses. | Wichtl 2004 with reference to Swiss Pharmacopoeia V. edition 1953 (no posology or indication) |

| Traditional use and therapeutic indication | **Posology** | **Reference** |
|---|---|---|
| Diaphoretic | 20 g (20 ml) as juice (Succus Sambuci inspissat') in port wine once daily. | Madaus 1938 |
| Diaphoretic | Herbal tea:<br>Single dose<br>1 teaspoon/cup of the herbal substance as an infusion or decoction. | Karmazín et al. 1984 |
| Diaphoretic | Roob Sambuci (elderberry syrup):<br>Fructus Sambuci recens 1000 T.<br>Aqua 200 T.<br>Saccharum q.s<br>Taken in large doses. | Wichtl 2004 with reference to Swiss Pharmacopoeia V. edition 1953 (no posology or indication) |
| Against cold and coughs | Roob Sambuci (elderberry syrup):<br>2.27 kg of fresh berries cooked with 453 g of sugar to the thickness of honey. One or two tablespoons mixed with a small glass of warm water and taken at night. | British Pharmacopoeia 1788 cited by Grieve 1931 |
| Against cold | Elderberry syrup:<br>Ripe berries and 14 g (1/2 oz) of whole cloves and ginger, boiling for an hour and strain. Add in hot water to a wineglassful of elderberry syrup and drinking. | British Pharmacopoeia 1788 cited by Grieve 1931 |
| Feverish catarrhal affections | 10 g dried fruit into cold water allow standing for several minutes and slowly heating to boiling; allow steeping for 5-10 minutes, and then decant.<br>As a tea:<br>One cup of tea several times daily (1 teaspoon = about 3.2 g). | Bisset and Wichtl 2001, Wichtl 2004 |
| Diuretic | Roob Sambuci (elderberry syrup):<br>Fructus Sambuci recens 1000 T.<br>Aqua 200 T.<br>Saccharum q.s<br>Taken in large doses. | Wichtl 2004 with reference to Swiss Pharmacopoeia V. edition 1953 (no specific posology or indication) |
| Diuretic | Herbal tea:<br>1 teaspoon/cup of the herbal substance as an infusion or decoction. | Karmazín et al. 1984 |
| Diuretic | 10 g dried fruit into cold water allow standing for several minutes and slowly heating to boiling; allow steeping for 5-10 minutes, and then decant.<br>As a tea:<br>One cup of tea several times daily (1 teaspoon = about 3.2 g). | Bisset and Wichtl 2001, Wichtl 2004 |

| Traditional use and therapeutic indication | **Posology** | **Reference** |
|---|---|---|
| Analgesic, sedative effects | 20 g (20 ml) as juice (Succus Sambuci inspissat') in port wine, once daily. | Madaus 1938 |
| Headache, dental, heart, nerve pain | Preparation of tea:<br>10 g fresh elderberry in cold water, leave for several minutes and then heat slowly to boiling. Let stand 5 – 10 minutes.<br>A cup of tea several times a day. | Hiermann 2010 |

Other uses mentioned:

| **Therapeutic indication** | Reference |
|---|---|
| Colic, diarrhoea and bronchitis | Grieve 1931 |
| Remedy for long-standing rheumatism, neuralgia and sciatica | Weiss 1988 |
| Sinusitis, chronic nasal catarrh with deafness and for local anti-inflammatory action | Ebadi 2001 |

Mild diuretic effect is reported in some handbooks such as Grieve 1931, Bisset and Wichtl 2001, Ebadi 2001 and Wichtl 2004. Mild diuretic effect is also mentioned in Karmazín et al. 1984 .

Laxative use is also mentioned in the British Pharmacopoeia 1788, Madaus 1938, Grieve 1931, Czech National Norm 1958, Bisset and Wichtl 2001, Wichtl 2004 and in Karmazín et al. 1984.

Elderberry has also been traditionally used against catarrhal conditions, especially in the upper respiratory tract such as cough, fever and colds and has been reported in Bisset and Wichtl 2001, Wichtl 2004 and Greive 1931 with reference to the British Pharmacopoeia 1788.

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

*Assessor's comments*

*Elderberry has been used both as a tea and decoction for many years in European countries like Germany, England, Czech Republic and Poland for various complaints. The requirement to show 30 years of medicinal use for an herbal preparation with a defined posology for a traditional indication is not fulfilled for elderberry. Only a single dose is described in the available sources of information. A specified daily posology for the herbal preparation is needed to fulfill the criteria for evidence of a traditional medicinal use for a specified posology.*

*The traditional use of elderberry syrup (Roob Sambuci) as a laxative according to the information cited in Wichtl 2004 with reference to the Swiss Pharmacopoeia and Grieve 1931 with reference to the British Pharmacopoeia 1788, is related to use of high doses and without specified posology.*

*None of the herbal preparations with indications such as laxative use, use against common cold, diaphoretic use and diuretic use listed in handbooks fulfills the requirements for inclusion in a monograph.*

# 3. Non-Clinical Data

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

**Pharmacodynamics**

***In vitro* experiments**

Immunological activity

Elderberry extracts (see Table 3) have been tested for immunomodulatory activity on monocytes from healthy individuals, and an increase in their cytokine production was observed *in vitro* following stimulation. The production of inflammatory cytokines was tested using blood derived monocytes from 12 healthy human donors *in vitro*. Elderberry extracts and lipopolysaccharid (as a positive control for monocyte activation) were added to the monocytes and incubated. The results show an increase in secretion of proinflammatory cytokines (tumour necrosis factor-alpha and interleukins IL-1β, IL6, and IL-8), and the stimulatory activity was dose dependent. E.Ex. (Standardised Elderberry Extract) showed the highest cytokine stimulation followed by B.E. (Black Elderberry Syrup – for composition see Table 3) and A.D. (Active Defense – for composition see Table 3) which contain the same amount (38%) of (E.Ex.) and had similar stimulatory effect on these cytokines (Barak et al. 2001).

**Table 3:** Elderberry preparations studied for immune-modulating activities (Barak et al. 2001)

| **Elderberry preparations** | **Ingredients** |
|---|---|
| Black Elderberry Syrup (B.E.) | 38% E.Ex., glucose, raspberry extract, citric acid and honey |
| Combination product (A.D.) | 38% E.Ex., glucose, raspberry extract, citric acid, honey, *Echinacea angustifolia*, *Echinacea purpurea*, propolis, ascorbic acid and zinc gluconate |
| Standardised Elderberry Extract (E.EX.) | 100% black elderberry extract |
| Elderberry syrup for Kids | 19% E.Ex., glucose, raspberry extract, citric acid, *Echinacea* |

Waknine-Grinberg et al. (2009) studied the immunomodulatory effect of standardised elderberry extract on leishmanial and malarial infections. A nontoxic dose of a standardised elderberry extract was examined in murine models of leishmaniasis and malaria. The elderberry extract causes a shift in the immune response, as demonstrated in human monocyte cultures, to Th1 (inflammation-associated) responses. Treatment of leishmania-infected mice with standardised elderberry extract delayed the development of the disease. As there was no direct *in vitro* anti-leishmanial effect, the observed partial protection *in vivo* is most likely related to immune modulation. Although increased Th1 responses are associated with protection from leishmaniasis, they are considered to be the main immunopathological processes leading to cerebral malaria. Administration of standardised elderberry extract to mice prior to and following infection with *Plasmodium berghei* ANKA increased the incidence

of cerebral malaria, while administration of standardised elderberry extract after infection had no effect on the disease. The results indicate how an inflammatory-like response may alleviate or exacerbate clinical symptoms of disease and hint at the importance of administration timing. The overall effect of depends on the ongoing immune response and the Th1/Th2 balance determined by both host and parasite defense mechanisms.

It has been reported that lectins are able to promote the release of histamine from basophils and mast cells. *S. nigra* agglutinin (unknown which) can cause allergic reactions because it is able to release interleukins IL-4 and IL-13 from human basophils and mast cells (Haas et al. 1999).

Förster-Waldl et al. (2003) examined the possible allergens in extracts from elderberry pollen, flowers and berries *in vitro*. Flowers, berries and pollen were ground in liquid nitrogen before extraction. Proteins were extracted 10% (w/v) in 10 mmol/L potassium phosphate buffer (pH 7.0). N-terminal sequence analysis of purified elderberry allergen indicated that the elderberry allergen is a ribosomal inactivating protein (RIP) called Sam n 1. It was reported that inhalative contact with elderberry pollen can cause an allergic reaction characterized by rhinitis and dyspnea in some patients who are allergic to grass pollen. These patients can possibly experience an allergic reaction to elderberry fruit. Only 0.6% of 3,668 randomly tested patients showed positive skin test to elderberry allergen.

Antiviral activity (influenza virus)

The inhibitory activity of a standardised elderberry extract has been studied in cell cultures infected with multiple strains of human influenza A and B type and animal influenza strain of this virus. Darby canine kidney cells were incubated with the standardised elderberry extract. It was shown that the standardised elderberry extract prevented virus to bind to host cell. This inhibition was attributed to the presence of novel flavonoids such as methylated flavonoid (5,7,3´,4-tetra-O-methyl-quercetin) and esterified flavonoid (dihydromyricetin-3-yl-3,4,5-trihydroxycyclohexanecarboxylate) (Zakay-Rones et al. 1995).

Roschek et al. (2009) studied the anti-influenza activity of an elderberry extract *in vitro*. The extract was obtained by extracting 20 g of ground elderberries using supercritical CO2, followed by two extractions using ethanol diluted with water (4:1, v/v). The major contributors to the anti-influenza activity were shown to be flavonoids (5,7,3´,4-tetra-O-methyl-quercetin) and dihydromyricetin-3-yl-3,4,5-trihydroxycyclohexanecarboxylate). These flavonoids bind to H1N1 virus particles which leads to inability of the H1N1 virus to enter host cells and thereby preventing virus infection. Based on these studies, it seems like elderberries contain antiviral flavonoids that inhibit the replication of common human and animal influenza A and B strains as well as prevent H1N1 viral infection *in vitro*.

Antioxidative activity

Elderberries contain anthocyanin flavonoids known to possess significant antioxidant properties. Low concentrations (4 µg/ml) of spray-dried elderberry juice can effectively renew $\alpha$-tocopherol from the $\alpha$-tocopheroxyl radicals in models of copper-mediated LDL oxidation. Anthocyanins such as cyanidin 3-glucoside and cyanidin 3-sambuboside which are the major antocyanins in the fruits may be responsible for this effect of the extract (Abuja et al. 1998).

A fruit juice concentrate made from fresh berries of Aronia (*Aronia melanocarpa*), elderberry (*S. nigra*) and macqui (*Aristotelia chilensis*), was tested for antioxidant activity *in vitro.* $H_2O_2$-induced DNA damage as well as oxidised DNA bases were determined in human tumour HT29 clone 19a cells. Two test methods were used, the micro gel electrophoresis assay (Comet test) which is a sensitive method to measurement of genotoxic effects and DNA damage, and ferric reducing ability assay (FRA assay) to examine intracellular oxidative/antioxidative effects of the samples. The results indicated that intracellular oxidative stress was little affected by natural plant ingredients such as anthocyanins and anthocyanidins. The mechanism behind these antioxidative effects are, however, not clear. In this FRA test, the antioxidative capacity may be a reflection of the compounds' potential to chelate $FeCl_3$ as well as to scavenge free radicals. Among anthocyanin concentrates, elderberry exhibited the strongest antioxidant activity followed by Aronia and macqui (Pool-Zobel et al. 1999).

Antibacterial activity

Extracts of raspberry, cranberry, elderberry, strawberry, bilberry, blueberry and a combination of these extracts were examined for their antibacterial activities against *Helicobacter pylori* with and without addition of clarithromycin. Antibacterial effects were observed of the various extracts at different concentrations (0.25%, 0.5% and 1%). The results indicated that inhibition of *H. pylori* is concentration-dependent and the combination seemed to have the greatest effect among the extracts tested. Experiments in which clarithromycin was added to the extracts showed greater inhibition of *H. pylori*. Since this is the first report of such activity, further studies are necessary for confirmation of the results (Chatterjee et al. 2004).

Hearst et al. (2010) investigated some Irish traditional herbal medicines for antibacterial activity against antibiotic-resistant common nosocomial pathogens. Freeze-dried powder formulations of elderberry showed activity against hospital bacteria methicillin resistant *Staphylococcus aureus* (MRSA) that become resistant to many conventional antibiotics. In this article, the antibacterial properties of elderberry were attributed to the major constituents including terpenes.

Lectin activities

Plant lectins are a heterogeneous group of proteins or glycoproteins that have a common unique ability to recognise specific sugar or sugar-containing macromolecules. Different lectins named *S. nigra* agglutinin (SNA) have been identified from different parts of elderberry, mainly in fruits, seeds and bark. These lectins are SNA-I, SNA-II and nigrin b (from the bark), SNA-III = SNA-IVf and SNA-Vf = nigrin f (from the fruits), and each one is slightly different in structure which leads to different sugar specificity (Van Damme et al. 1997, Girbes et al. 1996). SNA-II and SNA-III are both regarded as galactose-specific lectins (Peumans et al. 1991).

Nigrin f which is a ribosome-inactivating protein (RIP) consists of two different polypeptide chains, a catalytic A chain (toxic) and a B-chain (lectin). Nigrin f displays an N-glycosidase activity on the large ribosomal RNA of mammalian, plant and bacterial-sensitive ribosomes. Nigrin f is a non-toxic type 2 RIP (Citores et al. 1996, Van Damme et al. 1997).

Citores et al. (1996) examined the various activities of the lectins from crude extract (unripe/scratch) on rat *in vitro*. They analysed the effects of nigrin f on protein synthesis and translation. Protein

synthesis was carried out in cell free translation systems derived from rabbit reticulocyte lysates, rat liver, wheat germ, *Vicia sativa* germ, and *Cucumis sativus* bacterium. The result showed that nigrin f had an inhibitory effect on protein synthesis performed by rabbit reticulocyte lysates and rat liver cell-free systems, while it had no effect on plant cell-free systems derived from wheat, *V. sativa* and *C. sativus* bacteria. Nigrin f $IC_{50}$ value (concentration of the protein that promotes 50% inhibition of protein synthesis) was 1.8 ng/ml for rabbit reticulocyte lysates and 3.7 ng/ml for rat liver cell-free systems which indicates the low values. In addition it was shown that the maturation of elderberry fruit leads to a 10 times reduction of nigrin f content in green unripe fruit.

***In vivo* experiments**

Antioxidative activity

Bobek et al. (2001) also studied antioxidative activity. Rats with acute colitis were fed for a month with a diet containing 4% black elderberry extract (extraction solvent 70% ethanol, concentrated to the 50-60% content of dry matter.) Colitis was induced by intraluminal instillation of 4% acetic acid. Long-term feeding with a diet containing black elderberry extract showed increased resistance to experimentally induced colitis. Lower levels of oxidative stress in the experimental group compared with the control group who received laboratory control diet (without black elderberry extract) was observed. In addition, neutrophil infiltration caused by myeloperoxidase activity was significantly lower, while higher activity of lysosomal enzymes (acid phosphatase and cathepsin D) that are released during inflammation was observed in colonic tissue in the experimental group. Both macroscopic damage to the colon and myeloperoxidase activity was 50% lower in the treatment group compared with the control diet group. The results suggest that a diet containing black elderberry extract can increase the level of antioxidative defense of the organism and thus lead to increased resistance to induction of colitis. Rats with acetic acid-induced colitis placed on an elderberry diet had significantly less macroscopic damage and 50% lower myeloperoxidase activity scores than did rats in the control diet group. The antioxidative effects of glutathione are considered to be detoxification of degradation products resulting from lipid peroxidation and DNA damage. Elderberry extract with abundant content of beneficial flavonoids is thought to prevent damage to cellular components caused by reactive oxygen species.

Effects on influenza-like symptoms

Chimpanzees (n=4) given a syrup containing a standardised elderberry extract, either as a prophylactic treatment or as a symptom-dependent treatment, experienced fewer flu-like, upper respiratory ailments than the chimpanzees (n=4) that were given a placebo (sugar syrup). During the first fall and winter “flu season” of the study, five chimpanzees in the experimental group received 10 ml elderberry syrup daily, while five chimpanzees constituting a control group received sugar syrup. When chimpanzees in the experimental group exhibited flu-like symptoms, they received an increased dose of elderberry syrup, 15 ml, twice daily. During the six months trial period, the control group exhibited flu-like symptoms over a total of 39 days, whereas the experimental group had symptoms for a total of 12 days. During the second flu season, the chimpanzees were strictly treated symptomatically with 15 ml of elderberry syrup twice daily. Results from this study showed that chimpanzees given elderberry syrup as a prophylactic against influenza-like symptoms were 3 times

less likely to become ill than animals in the placebo group. In addition, chimpanzees in the experimental group that were treated with a triple dose of elderberry syrup when they exhibited symptoms of influenza during the course of the experiment were ill for fewer days (6-12) than the untreated animals in the control group. The effect of the standardised elderberry extract was investigated by visually observing symptoms of cold in the animals, during 3 periods of 15 minutes per day; the effect was shown in 4 animals. The results of this study indicate that a standardised elderberry extract may reduce flu and flu-like symptoms and reduce the duration of the disease (Burge et al. 1999).

*Assessor's comments*

*There is not enough scientific information available to conclude on an immunomodulatory effect or on an antibacterial effect of any of these elderberry extracts. An elderberry extract has shown inhibition of hemagglutinin and replication of common human and animal influenza A and B in vitro.*

*The antioxidative capacity of an elderberry extract has also been shown, but the mechanism is not clear. The major constituents, cyanidin 3-glucoside and cyanidin 3-sambubioside seem to be the most important antioxidants.*

*In vitro studies have shown that the ripening process leads to a significant reduction of the amount of lectin, and this is confirmed by the fact that ripe fruits are not toxic to humans.*

*Elderberry ethanol extract has been shown to protect rats against induced colitis.*

*A syrup containing a standardised elderberry extract has been shown to have symptomatic and prophylactic effects on four chimpanzees compared with four placebo treated chimpanzees.*

**Pharmacodynamics interactions**

No information available.

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

**Pharmacokinetics**

No data available.

**Pharmacokinetic interactions with other medicinal products**

In a study on rats, an aqueous extract of elderberries (decoction 1:10) administered orally (2 ml/kg) caused a reduction of the sleep induction time of pentobarbitone and a very modest increased sleeping time when compared with rats administered pentobarbitone only. No significant effect was observed on the analgesic activity of morphine taken in combination with elderberries. These preliminary experiments indicate an interaction between the elderberry and the centrally acting drug pentobarbitone (Jakovljevic et al. 2001). Evidence for an interaction between the extracts of elderberry and pentobarbitone appears to be limited to this study on rats which found only a very modest increase in sleep time. It is not known whether this effect will occur in humans, but even if it does, it is unlikely to be clinically relevant.

A combination product, sold as a food supplement containing *Echinacea purpurea* and *S. nigra*, was evaluated for the inhibitory potential of isolated human CYP3A4 with testosterone as substrate *in vitro*. The product performed relatively weak inhibition of CYP3A4 activity with an $IC_{50}$ value (half maximal inhibitory concentration) of 1.192 mg / ml. The inhibitory potency seems to be exercised by *E. purpurea* whereas *S. nigra* showed an insignificant inhibitory effect on CYP3A4 (Schrøder-Aasen et al. 2012).

*Assessor's comments*

*There is not enough data available to draw conclusions about the potential effect of elderberry on centrally acting drugs such as morphine and phenobarbital. Elderberry did not show inhibitory effect on CYP3A4 in vitro.*

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

**Toxicology**

Cyanogen glycosides and lectins

The major cyanogenic glycosides are mainly found in the edible parts of plants. When these plant parts are consumed, ß-glucosidase that is present in the gut comes in contact with glycosides and cyanogenic glycosides hydrolyze and produce hydrogen cyanide (HCN) which is toxic (JECFA 1993). Elderberry leaves have been proved to contain cyanogenic glycosides such as zierin, Sambunigrin, Prunasin and Holocalin (Jensen and Nielsen 1973, Dellagreca et al. 2000).

Dewick (2009) also provides information about the crushing of the plant containing cyanogenic glycosides. The author indicated that the crushing leads to glycosides come in contact with glycosidase present in the plant and then release of HCN.

Several sources, summarised in the systematic review by Vlachojannis et al. (2010), warn that all plant parts of elder containing cyanogenic glycosides including unripe/uncooked fruits, fresh leaves, bark and roots can cause nausea, vomiting or severe diarrhoea when consumed and should be avoided in elderberry preparations (Duke et al. 1985, Bisset and Wichtl 2001, Lewis and Elvin-Lewis 2003, Vallès et al. 2004). The presence of small amounts of HCN in fruit seeds is the only concern of elderberry preparations, but these toxic substances are removed by heat treatment or cooking of berries/juice since HCN is volatile and evaporates (Pogorzelski 1982).

According to Battelli et al. (1997), bark lectins, mainly nigrin b, are toxic because of their ability to bind to eukaryotic cells and terminate protein synthesis. Nigrin b has structural and activity similarities to ricin but has been shown to be less toxic. Gayoso et al. (2005) also reported that nigrin b is less toxic to animal cells cultures than ricin.

In May 2012, the European Food Safety Authority (EFSA) published a new version of the compendium of plants reported to contain toxic, addictive, psychotropic or other substances from 2009, "Compendium of botanicals reported to contain naturally occurring substances of possible concern for

human health" (http://www.efsa.europa.eu/en/efsajournal/pub/2663.htm). *Sambucus nigra* L. has been listed with the following information:

**Table 4:** Information about *Sambucus nigra* L. in EFSA Compendium of botanicals 2012

| | |
|---|---|
| Botanical name | *Sambucus nigra* L. |
| Family | Adoxaceae (Caprifoliaceae) |
| parts of plants of possible concern | Whole plant |
| Chemical of concern | Cyanogenic glycoside: S-sambunigrin (3 to 17 mg HCN /100 g fresh weight in leaf and 3 mg HCN / 100 g of fruit) |
| Remarks on toxic/adverse effect(s) not known to be related to the identified chemical(s) of concern | Presence of lectins in branches, unripe berries or seeds of a number of different Sambucus species induce gastrointestinal disorders |
| Specific References | Sangiorgi, E., Minelli, E., Crescini. G. and Garzanti, S. (2007) Fitoterapia. (Ed. Casa Editrice Ambrosiana). ISBN: 978-8808-18266-1<br>Frohne D., Pfänder H.J. and Anton R. 2009. « Plantes à risques », Ed. Tec et Doc Lavoisier, ISBN : 978-2-7430-0907-1<br>EMEA HMPC. 2007. *Sambucus nigra* L., flos - Assessment report for the development of community monographs and for inclusion of herbal substance(s), preparation(s) or combinations thereof in the list. EMEA/HMPC/283170/2007/Corr |

Acute toxicity

Non information available.

Repeated dose toxicity

No information available.

Reproductive and developmental toxicity studies

No reproductive and developmental toxicity studies have been found.

Genotoxicity

There are no data on genotoxicity available on elderberry.

## *3.4. Overall conclusions on non-clinical data*

**Pharmacology - Pharmacodynamics**

*In vitro* experiments showed that elderberry extract inhibited the replication of common human and animal influenza A and B strains as well as prevention of viral adhesion of two cell receptors. The studies showed that methylated and esterified flavonoids may contribute to the antiviral activity. There

is not enough evidence to suggest an immune-modulatory effect of elderberry extract. No studies are available that can support the laxative effect of elderberry. No data are available that substantiate beneficial effects related to lectins. A small *in vivo* experiment with four chimpanzees indicated that flu-like symptoms were reduced after treatment with elderberry syrup in the experimental group.

**Pharmacokinetics**

There is not enough data available to draw conclusions about elderberry potential effect on the pharmacokinetic parameters of the centrally acting drugs morphine and pentobarbitone.

**Toxicology**

Toxicological data on elderberry is limited. Nonetheless, neither the chemical composition nor the long-term widespread use as food in the European Union (see section 5.2.) suggests that there is a high risk associated with the use of elderberry preparations prepared from ripe fruits. Non-clinical data indicate no signals of toxicological concern when the preparations are based on cooked or heat treated products. Elderberry bark and all plant parts containing bark (branches) should be avoided in elderberry preparations.

# 4. Clinical Data

## *4.1. Clinical Pharmacology*

No data available.

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

No data available.

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

Pharmacokinetics

Mülleder et al. (2002) recruited 16 healthy volunteers (8 men, 8 women) to a pharmacokinetic study of elderberry cyanidin-3-glucoside and cyanidin-3-sambubioside. The main purpose of this study was to identify possible metabolites of anthocyanins from elderberry in urine and quantify the biokinetics of these compounds. The volunteers had ingested 11 g elderberry concentrate (1.9 g of anthocyanins equivalent to 235 ml fresh juice) one day diluted with water, the other day with 30 g of sucrose together with the elderberry juice. Urine samples were taken before and over a period of 6 hours with intervals of 1 hour after ingestion. The results showed that anthocyanins were absorbed from the intestine to the blood, and very low recoveries were found in urine (0.003–0.012% of the oral dose) 4 hours after intake. The addition of sugar to elderberry juice resulted in a delay of excretion suggesting that intestinal sugar carriers may play a role in flavonoid absorption. The anthocyanins can be absorbed into the enterocyte by its glucose moiety, which is bound by a glucose transporter. It is possible that consumption of sugar leads to a saturation of the glucose transporter and inhibiting the

uptake of anthocyanins. Possible metabolites were observed in this study but not identified (Mülleder et al. 2002).

Four women (age: 67 ± 4 years) were recruited to a pharmacokinetic study. Subjects were fasted overnight before the day of baseline blood sampling. Each subject was then given 12 g elderberry extract containing 720 mg anthocyanins, mainly cyanidin-3-sambubioside, cyanidin 3-glucoside, and maltodextrin dissolved in 500 ml water. Blood samples (15 ml) and urine samples were collected from these subjects before and after consumption.The results showed that anthocyanins (which are glycosides) seems to be hydrolysed in the gut so that only the aglycones (anthocyanidins) were absorbed within 72 minutes and metabolised in the liver by glycosylation to increase the solubility prior to excretion via the kidneys. The elimination of plasma anthocyanins appeared to follow first-order kinetics and most anthocyanin compounds were excreted in urine within 4 hours after ingestion (Milbury et al. 2002).

One male subject 35 years of age consumed 25 g of elderberry extract as one dose, containing 1.5 g anthocyanins after fasting overnight. Blood samples were collected before and 30 and 60 minutes after anthocyanin consumption. Cyanidin-3-glucoside and cyanidin-3-sambubioside were detected in plasma indicating that anthocyanins can be absorbed in their glycosidic forms in humans (Cao and Prior 1999).

Absorption and metabolism of anthocyanins of elderberries were studied in four healthy women, 60-70 years. They consumed 12 g elderberry extract that contained a total of 720 mg of anthocyanins mixed in 500 ml water after overnight fasting. Urine samples were collected before and after the consumption of elderberry extract. Two major anthocyanins cyanidin-3-glucoside and cyanidin-3-sambubioside, and four metabolites: 1) peonidin-3-glucoside, 2) peonidin-3-sambubioside, 3) peonidin monoglucuronide, and 4) cyanidin-3-glucoside monoglucuronide were identified in the urine within 4 hours after ingestion. This study suggests low rates of absorption and excretion of anthocyanins compared with other flavonoids (Wu et al. 2002).

*Assessor's comments*

*The bioavailability seems to be very low in the above mentioned studies.*

**Table 5:** Bioavailability studies on elderberry (*S. nigra* L.)

| Subject | Design | Dosage | Preparation | Results/Conclusion | References |
|---|---|---|---|---|---|
| Detection of anthocyanins in human plasma | n=1 healthy male (35 years) | 25 g extract taken as one dose | Elderberry extract (containing 1.5 g anthocyanins) | Anthocyanins cyanidin-3-glucoside and cyanidin-3-sambubioside were detected. | Cao and Prior 1999 |
| Urinary excretion of cyanidin glycosides | O, CO n=16 (8 men, 8 women) | 11 g concentrate a day diluted with water, the other day with | Elderberry concentrate (equivalent to 235 ml of fresh juice containing 1.9 g anthocyanins) | 2 main anthocyanins detected unchanged in urine at low concentration. Ingestion of sucrose reduced excretion. | Mülleder et al. 2002 |

| | | | | | |
|---|---|---|---|---|---|
| | | 30 g sucrose. | | | |
| Detection of anthocyanins in human plasma and urine | O n=4 elderly women (63-71 years) | 12 g | Elderberry extract (containing 720 mg anthocyanins) dissolved in water | Detection of 2 primary anthocyanins as glycosides in plasma and urine. | Milbury et al. 2002 |
| Absorption And metabolism of anthocyanins | O n=4 elderly women (60-70 years) | 12 g | Elderberry extract (containing 720 mg anthocyanins) dissolved in ater | Study suggests low rates of absorption and excretion of anthocyanins compared with other flavonoids | Wu et al. 2002 |
| KEY: CO – crossover, n – number of patients, O – open | | | | | |

## *4.2. Clinical Efficacy*

### 4.2.1. Dose response studies

There are no dose response studies available

### 4.2.2. Clinical studies (case studies and clinical trials)

Two clinical studies have been conducted by Zakay-Rones et al. (1995, 2004) studying the safety and efficacy of a syrup containing a standardised elderberry extract in the treatment of influenza and its symptoms.

The first study was conducted on 27 people (15 receiving treatment and 12 receiving an undefined placebo preparation). Improvement of flu symptoms including fever was obtained in 93.3% of the patients within 2 days after initial dosing, while 91.7% in the placebo group ($P<0.001$) showed signs of improvement after 6 days. Immunological tests found a higher level of influenza virus antibodies in patients who received elderberry than those who received the placebo, suggesting an enhanced immune activity. None of the patients reported any adverse reactions related to the medication (Zakay-Rones et al. 1995).

The second study was conducted in 60 people (30 were treated with and 30 were given an undefined placebo preparation) with early symptoms of flu. The treatment effect (15 ml four times a day) was evaluated by assessing symptoms and general wellbeing and the result was determined by visual analog score (VAS) self-measuring flu symptoms (such as pain, cough frequency, sleep quality, nasal congestion, and mucous discharge) and self-assessment questionnaires. There was a significant difference between the two groups in the development of mean VAS scores. VAS values were significantly higher (showing an improvement) in the treatment group than the placebo group. Most

VAS values in the elderberry group were close to 10 which was the highest score (pronounced improvement) after 3-4 days of treatment, while it took 7-8 days before the placebo group reached similar levels. The use of rescue medication (analgesic and nasal spray) was significantly lower in treatment group ($P < 0.001$). Symptoms were relieved on average 4 days earlier and use of rescue medication was significantly less in those receiving elderberry extract compared with placebo. None of the patients reported any adverse reactions related to the medication (Zakay-Rones et al. 2004). This study was sponsored by the producer of this commercial extract.

A randomised, placebo-controlled study on 34 healthy subjects examined the effectiveness of low-dose, powdered elderberry juice (10% anthocyanins) versus placebo on lipid parameters. Elderberry was dosed at 400 mg capsuled powder (equal to 5 ml elderberry juice) three times daily for two weeks; patients were instructed to follow a diet containing 35% fat. Analysis of results showed a slight, but statistically insignificant decrease at two weeks in all lipid parameters of the low-dose elderberry extract group compared to baseline. Total cholesterol was 199 mg/dl at baseline versus 190 mg/dl at the end of the two-week period. Although improvements in lipid values were statistically insignificant, the dosage of elderberry extract was low and it is possible that higher dosages may produce a more significant benefit. In addition, subjects with normal lipid levels may not be able to obtain significant reductions since their lipids are already within the normal range. Further studies on patients with elevated lipid levels are necessary. Prior to this study a short-term open study was performed as pilot study as shown in Table 6 (Murkovic et al. 2004).

**Table 6**: Clinical Studies on elderberry (*S. nigra* L.)

| Subjects | Study design | Duration | Dosage | Preparation form | Results | Researcher |
|---|---|---|---|---|---|---|
| Influenza treatment | DB, PC, R n=27 15 receiving treatment and 12 receiving an undefined placebo preparation | 3 days | Children: 2 table spoons daily Adults: 4 table spoons daily | Black elderberry extract containing anthocyanins | Enhanced immune activity detected in the treatment group | Zakay-Rones et al. 1995 |
| Influenza treatment | DB, PC, R n=60 30 treated and 30 were given an undefined placebo preparation | 5 days | 15 ml, 4 times a day | Black elderberry extract containing anthocyanins | Improvement in symptoms using a visual analogue scale (VAS) occurred after 3 days in treatment group, and after 7 days in placebo group | Zakay-Rones et al. 2004 |

| Subjects | Study design | Duration | Dosage | Preparation form | Results | Researcher |
|---|---|---|---|---|---|---|
| Serum cholesterol | O, PC, R n=34 20 men, 14 women | 2 weeks | 400 mg in capsules 3 times daily | Spray-dried powder containing 10% anthocyanins equivalent to 5 ml elderberry juice | Non-significant decrease in total cholesterol concentrations in treatment group compared with placebo group | Murkovic et al. 2004 |
| KEY: n – number of patients, O – open, R – randomised, DB – double blind, PC – placebo controlled | | | | | | |

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

No information available.

### *4.4. Overall conclusions on clinical pharmacology and efficacy*

Three controlled clinical studies have been conducted to determine the effectiveness of herbal preparations of elderberry, with very small numbers of patients. Two of the clinical trials studied the effectiveness of elderberry on flu treatment and the third one on blood lipids reduction. Results from two clinical studies indicate possible effectiveness of elderberry aqueous extract for treatment of influenza suggesting a faster recovery. More studies are needed to confirm this effect.

Effect of powdered elderberry juice on blood lipids showed insignificant reduction of blood lipids which may be due to the low dose of elderberry extract. It could be possible that higher doses may cause more significant effect on lipid reduction. Further studies are needed.

There are no clinical investigations available on elderberry laxative activity. Overall the existing data cannot be considered to meet the criteria for "well-established medicinal use" in accordance with Directive 2001/83/EC.

## 5. Clinical Safety/Pharmacovigilance

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

### *5.2. Patient exposure*

A considerable patient/consumer exposure must be anticipated as elderberry is widely used as a natural source of food flavouring and in food supplements on the market in Europe. The regulatory status implies that no systematic pharmacovigilance activities have been performed.

## 5.3. Adverse events and serious adverse events and deaths

The World Health Organization Uppsala Monitoring Centre (WHO-UMC) received a report from the national pharmacovigilance centre of Canada after a search (conducted 9/27/2011). Abdominal pain after oral administration of elderberry syrup by a 48-year-old woman was reported. The national pharmacovigilance centre of Norway reported two cases, one knee pain in a 75-year-old man and an increased International Normalized Ratio (INR) in a 75-year-old woman after ingestion of an aqueous elderberry extract as a dietary supplement (see Table 7). The national pharmacovigilance center of Australia reported to WHO-UMC a total of 26 adverse events to the combination product containing *Andrographis paniculata*, *Salix alba* and *S. nigra*. Among those 26 adverse events, 22 were described as various allergic reactions.

Acute poisoning was reported after ingestion of juice made from berries crushed with their leaves and branches of *S. mexicana*, the elder tree indigenous to the western United States. Within 15 minutes after drinking the juice, 11 people experienced nausea and vomiting. One report of severe illness following the ingestion of juice prepared from elderberries has been recorded by the Centres for Disease Control in 1984. People attending a picnic, who ingested several glasses of juice made from berries, picked the day before, reported nausea, vomiting, weakness, dizziness, numbness, and stupor. Eleven people experienced nausea and vomiting, eight of whom had acute GI and neurologic symptoms after ingesting an elderberry juice made from raw elderberries, leaves, and branches. One person who consumed 5 glasses of juice was hospitalized for stupor. All recovered. The poisoning was attributed to the content of a bitter alkaloid and cyanogen glycosides in leaves, bark and buds that were crushed together with the fruits in the production of the juice. The toxic glycosides may under certain circumstances liberate hydrogen cyanide (Kunitz et al. 1984).

**Table 7:** Adverse events

| **Country** | **Patient characteristics** | **Reaction(s)/Event(s)** | **Product** |
|---|---|---|---|
| Canada | Female 48 years | Abdominal pain | Sambucus nigra (elderberry syrup) |
| Norway | Male 75 years | Oedema legs | Sambucus nigra (as food supplement) |
| Norway | Female 75 years | INR decreased | Sambucus nigra (as food supplement) |

## 5.4. Laboratory findings

No information available.

## 5.5. Safety in special populations and situations

Use in children and adolescents

The use is not recommended in adolescents and children below 18 years due to insufficient data on safety and efficacy.

Drug interactions

Theoretically, elderberry might interfere with immunosuppressant therapy because of possible immunostimulating activity. Elderberry may stimulate the production of cytokines from human monocytes even though this must be confirmed in studies of higher quality. Immunostimulating herbal remedies may interfere with immunosuppressant drugs and corticosteroids (Barak et al. 2001).

Use in pregnancy and lactation

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended because of potential risk of toxicity.

Overdose

None reported.

Drug abuse

None reported.

Withdrawal and rebound

None reported.

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

No studies on the effect on the ability to drive and use machines have been performed.

### *5.6. Overall conclusions on clinical safety*

Plant parts other than the flowers and ripe berries are reported to be poisonous and should not be ingested nor be present in herbal preparations with elderberry. Hence, inclusion of other parts of the plant should be avoided. Bark lectins are considered to be toxic and should be avoided in elderberry preparations. In addition, the leaves and stems should not be crushed, since crushing leads to the liberation of hydrocyanic acid.

Heat treatment of fruit/juice will also reduce the small amount of hydrogen cyanide that can be liberated from the seeds and is therefore recommended. The fruits are safe to consume when ripe, cooked or dried. The safety of elderberry during pregnancy and lactation has not been established. Elderberry cannot be recommended during pregnancy or lactation and in children and adolescents under 18 years of age due to insufficient data on safety and efficacy.

## 6. Overall conclusions

Elderberry preparations have been studied in clinical trials, but the published clinical studies cannot be considered to fulfill the criteria required for “well-established medicinal use” according to Directive 2001/83/EC.

For elderberry preparations, complete information on traditional use with a specified posology is missing in order to establish a monograph that fulfills the requirements of medicinal use for at least 30 years (including at least 15 years within the European Union) according to Directive 2004/24/EC.

Based on the available information, a Community herbal monograph on *Sambucus nigra* L., fructus cannot be established at present.