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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Fragaria vesca* L., *Fragaria moschata* Weston, *Fragaria viridis* Weston and *Fragaria x ananassa* (Weston) Duchesne ex Rozier, folium

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Fragaria vesca* L., *Fragaria moschata* West., *Fragaria viridis* West. and *Fragaria x ananassa (*West.) Duchesne ex Rozier *folium*
Herbal preparation(s)	Comminuted herbal substance
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use
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1. Introduction

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

- Herbal substance(s)

Wild strawberry belongs to the genus *Fragaria* and it grows spontaneously throughout Europe, North America and North Asia. Wild strawberry images were commonly shown in medieval paintings (Sillasoo, 2006). The existing species of *Fragaria* are estimated to have last shared a common ancestor between 1.0 and 4.1 million years ago (Liston *et al*. 2014).

The genus *Fragaria* consists of at least 20 recognised wild species with different chromosome numbers (ie. 2x, 4x, 6x and 8x) (Hummer *et al*. 2011). After analysis of chloroplast DNA it was found that *Fragaria vesca* is the closest diploid (2x) relative to the cultivated octoploid strawberry *Fragaria ananassa* (8x) (Sargent *et al*. 2009).

Diploid *Fragaria vesca* L. was cultivated since the Middle Ages in European gardens, together with two other wild species with different chromosome numbers found in European forests: hexaploid *F. moschata* L., diploid *F. viridis* Duchenne and a form of the so-called "semperflorens or alpine" (Darrow 1966). The cultivated strawberry originated in France around 1750 from a random hybridisation of two wild American octoploid (8x) species which were identified by Antoine Duchesne in 1766 as *Fragaria chiloensis* L. and *Fragaria virginiana* Duch (Staudt 1951). For the next 100 years the cultivars originating from this hybridisation displayed a wide variety in morphology and seasonal versality (Gündüz 2016; Mishra *et al* 2015). The intraspecific variation in ploidy is not known, and therefore the chromosome number is a reliable way to differentiate between *Fragaria* species by use of genetic barcoding (Lundberg *et al*. 2009; Rousseau-Gueutin *et al*. 2009). Morphological diversity of the wild *Fragaria* flowers, sympodial stolons, achenes and leaves are characteristic of this species. Comparative taxonomic morphological and anatomical studies of collected leaves of several *Fragaria* species in various parts of Austria were performed by Scheller (2014). It was shown that different species of *Fragaria* differed only in stomata dimensions on the abaxial leaf surface and are hardly distuingishable.

Fragariae folium is defined in the Austrian Pharmacopoeia (Österreichisches Arzneibuch 2013, also DAC 2004) as “The collected, dried leaves of *Fragaria vesca* L., *Fragaria moschata* Weston, *Fragaria viridis* Weston, *Fragaria x ananassa* (Weston) Duchesne ex Rozier or a mixture of these species”. Content: at least 3.0 percent of tannins, expressed as pyrogallol ($C_6H_6O_3$; Mr 126.1) and based on the dried drug.

F. vesca leaves may contain about 5–11.4% of condensed tannins, flavonoids (quercetin, rutin), organic acids, glycosides, traces of essential oils and mineral salts (Hensel 2008; Lamaison *et al*. 1990, Wichtl and Bisset 1994). Phytochemical studies show a similar chemical profile for these species which can be distinguished by the use of genetic barcoding (Schneider, 1974; Blaschek *et al*., 2006; Scheller, 2014). Thus, traditional use in the European Union may be considered for all these species and the following Assessment Report and the corresponding Monograph refer to the scientific literature data on *Fragaria vesca* L., *Fragaria moschata* Weston*, Fragaria viridis* Weston*, Fragaria x ananassa* (Weston) Duchesne ex Rozier or a mixture of these species.

Common wild strawberry is a perennial plant of the Rosaceae family which grows in meadows, woods and along roadsides (Mabberley 2002). The plant is native from the west of the Ural Mountains across Northern Europe and North America. The present *Fragaria* taxonomy includes 20 named wild species which are distributed in the northern temperate and Holarctic zones (Hummer *et al*. 2011). It is a perennial plant of about 5-20 cm height. Leaves are complex (three-lobed, feathery) and have

serrated edges and hairy petioles. Wild strawberry produces stolons and is flourishing and bearings fruits for a short time, usually in the late spring and summer (Dhole *et al*. 2014). The wild strawberry leaves with petioles or without them, can be collected throughout the growing season. Wild strawberry grows in woods, forest glades and wood margins and it is a light-demanding species (Labokas and Bagdonaite 2005).

Elsewhere it is reported that wild strawberry leaves are collected during the flowering period for use in European traditional medicine, mainly prepared as a herbal tea as diuretic and to treat diarrhoea. Although cultivars of the wild strawberries are popular, due to their aromatic fruits, the commercial supply of wild strawberry leaf is for the most part wild harvested by rural populations in Albania, Bulgaria, Croatia, Kosovo, Serbia, North Macedonia and Ukraine. Wild strawberry leaf is at present exported to North America where it is used as a component of notified and licensed products in the USA and in Canada (Medicinal Plants and Natural Ingredients, 2015).

Chemical constituents:

Fragaria vesca L. leaves contain phenolic acids, quercetin and quercitrin, 2.2% rutin as well as catechin and ellagitannins, including pedunculagin, together with 5-11% condensed tannins (oligomeric proanthocyanides) (ARS 2016a, Blaschek *et al*. 2006, Hanhineva et al. 2011, Hiller and Melzig 2003, Schönfelder and Schönfelder 2004, Van Wyk and Wink 2004, Wagner 1999, Wichtl 1994; 2004).

The species *F. moschata, F. viridis* also contain a percentage around 3.4% of phenolic acids and 2.3% rutin, together with tannins (Blaschek *et al*. 2006).

Phenolic acids

Phenolic acids are found in both *Fragaria vesca* (salicylic acid, cinnamic acid, caffeic acid and chlorogenic acid) and *Fragaria viridis* (gallic acid, cinnamic acid, caffeic acid and chlorogenic acid) leaves (Blaschek *et al*. 2006).

Haghi and Hatami (2010) studied the content of phenolic acids in the wild strawberry leaves and found as mean content for unhydrolysed ellagic acid 1.72 mg/g and for hydrolysed ellagic acid 21.66 mg/g in dry samples of *Fragaria* leaves.

In the methanolic extract of *Fragaria vesca* after field-grown leaves-direct extraction (Yildirim and Turker 2014) the presence of following phenolic compounds was estimated by use of liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS): gallic acid monohydrate, pyrocatechol, procyanidin B1, (−) epigallocatechin, (+) catechin, procyanidin B2, vanillic acid, caffeic acid, procyanidin C1, (−) epicatechin, p-coumaric acid, (±) taxifolinhydrate, coumarin, luteolin-7-O-D-glucoside, rutin hydrate, resveratrol, myricetin, kaempferol-3-d-glucopyranoside, daidzein, quercetin, genistein, apigenin. Najda and Dyduch (2009a) and Dyduch and Najda (2009) stated, that leaves of cultivated species (*Fragaria vesca* “Regina”) contained less phenolic acids compared to the wild forms with a variation from 1.3% to 1.8%.

Flavonoids

Haghi and Hatami (2010) studied the content of flavonoids in wild strawberry leaves and found as mean content for quercetin 2.16±0.0 mg/g and for kaempferol 0.34±0.02 mg/g in dry samples of *Fragaria* leaves. Najda and Dyduch (2009a), Dyduch and Najda (2009) reported that leaves from cultivated species (*Fragaria vesca* “Regina”) contained more flavonoids (4%) as compared to the herb collected from natural habitats (3%). From a study performed in the western Carpathian Mountains, it is reported that *Fragaria vesca* occurs in a variety of habitats with variation in some measured parameters (number of leaves, length of the longest leaf, dry weight and total flavonoid content). The

flavonoid contents in the leaves were overall decreasing from lower to higher altitudinal zones (Pearson correlation coefficient R=0.770) (Malinikova *et al*. 2013).

Condensed tannins

Ivanov *et al.* (2015) found, that the most convenient method for the extraction of procyanidins (B1, B2 and B5) from *Fragaria vesca* leaves was a 56% acetone-water solvent with 50 min of ultrasonic extraction (frequency 35 kHz). They obtained a maximum amount of procyanidins 124.0 mg/100 g dry biomass.

Ellagitannins

In *Fragaria vesca* leaves and flowers following ellagitannins were found: agrimoniin, pedunculagin, other monomeric ellagitannins (e.g. casuarictin, agrimonic acid A/B, isostrictinin/sanguiin H-4), and other oligomeric ellagitannins (e.g. laevigatin isomers). The total content of ellagitannins in a hydro-methanolic crude extract (based on UV spectra) was reported as 51–89 mg/g (Moilanen *et al*. 2015). Using another assay, Oktyabrsky *et al*. (2009) reported a total tannin content of 8.2 mg/g (dry weight) in *Fragaria vesca* leaves ethanol extract.

Both, thin layer chromatography (TLC) and liquid chromatography (LC) methods, were used for the determination of polyphenols: agrimoniin, catechin, pedunculagin, ellagic acid and gallic acid in selected herbal medicinal products, Fragariae vescae folium included (Table 1, Fecka 2009).

Table 1: Contents of investigated polyphenols in *Fragaria vesca,* folium (Fecka 2009)

Mean content±SD, % (w/w)[a]						
	Product No.	**Gallic acid**	**Pedunculagin**	**Catechin**	**Ellagic acid**	**Agrimoniin**
Fragariae folium	Fv1	0.11±0.01	0.40±0.01	0.21±0.04	0.20±0.02	1.11±0.03
	Fv2	0.16±0.01	0.68±0.02	0.25±0.02	0.23±0.03	0.89±0.03

[a] n=3

Liberal *et al*. (2015) tested an ellagitannin-enriched fraction (EEF) from the ethanol and hydroalcoholic extracts of *Fragaria vesca* leaves. They identified 13 elagitannins: sanguiin H-10 isomer, castalagin/vescalagin isomer, sanguiin H-2 isomer, castalagin/vescalagin isomer, sanguiin H-10 isomer, sanguiin H-2 isomer, casuarictin/potentillin isomer, sanguiin H-6/agrimoniin, lambertianin A isomer and several unknown ellagitanins.

Terpenes

Hampel *et al*. (2006) determined by GC-MS analysis in *Fragaria vesca* leaves the terpene (R)-linalool with a high enantiomeric purity (>90%). The chromatographic analysis revealed that myrthenol, nonal, linalool and phtalide dibuthyl dominated in the essential oil obtained from the leaves of wild strawberry cultivars (“Rugia” and “Baron von Solemacher”) (Najda and Dyduch 2009b). Depending on the cultivar, the air-dried leaves contained from 0.46% (“Baron von Solemacher”) to 0.62% (“Rugia) of essential oil. The GC/MS analysis demonstrated the presence of 70 compounds in the leaves of “Rugia” and 58 compounds in the leaves of “Baron von Solemacher”.

2-pyrone-4, 6-dicarboxylic acid

During metabolic degradation of aromatic compounds and phenolics, first protocatechuic or gallic acid is formed followed by 2-pyrone-4.6-dicarboxylic acid. Wilkes and Glasl (2001) found that this

substance has an important taxonomic significance for the family of Rosaceae. The content of this substance in *Fragaria vesca* is 214 mg/100 g, and in *Fragaria ananassa* 239 mg/100 g.

- Herbal preparations

The content of phenolic compounds and their concentration contained in the aqueous extract of *Fragaria vesca* leaves was determined by the Folin-Ciocalteau colorimetric method (Mudnic *et al*. 2009) (Table 2).

Table 2: Identified phenolic compounds and their concentrations in the aqueous extracts of wild strawberry (*Fragaria vesca*, L.) leaves (Mudnic *et al*. 2009)

Compound concentration (mg/l) *Fragaria vesca*, L.	
(+)-Catechin	245.72
(-)-Epicatechin	259.36
Epigallocatechin	325.98
Procyanidin B1	175.06
Procyanidin B2	14.80
Epicatechin-3-gallate	120.50
Quercetin-4'-glucoside	39.93
Piceid	6.64
Astringin	165.04
Trans-Resveratrol	1.95

The phytochemical profile of *Fragaria vesca* leaves was studied by Liberal *et al*. (2012). HPLC-PDA phenolic profiles were recorded at 280 nm. The hydroalcoholic extract contained proanthocyanidins, flavonols, and ellagic acid and its derivatives. In total, 20 compounds were identified by HPLC-PDA-ESI/MSn.

Dias *et al*. (2015a, 2015b) studied the chemical composition of infusion and decoction of *Fragaria vesca* vegetative parts (leaves and stems) (Table 3 and Table 4).

Table 3: Minerals, soluble sugars, vitamins and organic acids in infusions and decoctions prepared from vegetative parts of *Fragaria vesca* L. samples (mean±SD) (Dias *et al*. 2015a)

	Leaves and stems
	Infusion
Ash content (g/100 mL)	0.24±0.0
Microelements (mg/100 mL)	
Fe	10 ± 1^{d}
Cu	nd
Mn	70 ± 1^{b}

	Leaves and stems
	Infusion
Zn	10±1[c]
Macroelements (mg/100 mL)	
Ca	6.5±0.2[c]
Mg	4.2±0.2[b]
K	1.26±0.03[c]
Soluble sugars (g/100 g)	
Xylose	5.82±0.07[a]
Fructose	6.4±0.1[b]
Glucose	7.42±0.01[c]
Sucrose	8.53±0.05[a]
Trehalose	3.56±0.02[a]
Sum	31.7±0.2
Vitamin C (Ascorbic acid, mg/100 ml)	Not detected
Vitamin B9 (Folate, mg/100 ml)	11.7±0.7[c]
a-Tocopherol (mg/100 ml)	0.22±0.01[b]
Oxalic acid	2.51±0.01[a]
Quinic acid	4.56±0.08[a]
Malic acid	1.8±0.1[c]
Shikimic acid	Not detected
Citric acid	1.08±0.06[c]
Fumaric acid	Not detected
Sum	9.99±0.03[d]

nd - not detected; Fe-iron Cu-cooper, Mn-manganese, Zn-zinc, Ca-calcium, Mg-magnesium, K-potassium. In each row different letters mean significant differences between samples ($p<0.05$), where "[a]"and "[b]" correspond to the highest and lowest values, respectively.

Table 4: Phenolic compounds quantification/estimation (mg/g) in water extracts prepared from *F. vesca* vegetative parts (Dias *et al*. 2015b)

Compound	**Infusion**	**Decoction**
Bis-HHDP-glucose[B]	1.72±0.12	0.79±0.21
(Epi)catechin hexoside[A]	4.51±0.09	2.02±0.18

Compound	Infusion	Decoction
Bis-HHDP-glucose[B]	0.63±0.06	0.79±0.09
rocyanidin dimer[A]	8.47±0.29	5.75±0.08
B-type (epi)catechin trimer[A]	4.82±0.16	2.85±0.23
Quercetin hexose glucuronide[E]	4.04±0.08	3.35±0.05
(+)-Catechin	21.65±0.01	15.39±0.08
B-type (epi)afzelechin-(epi)catechin[A]	5.53±0.04	3.58±0.56
Procyanidin dimer[A]	2.68±0.21	2.42±0.09
Quercetin deoxyhexose glucuronide[E]	15.21±0.08	13.57±0.01
Quercetin 3-O-rutinoside	5.11±0.12	4.23±0.02
Sanguiin h10 isomer[B]	7.40±0.11	3.51±0.05
Kaempferol deoxyhexose glucuronide[G]	11.96±0.07	9.21±0.05
Quercetin O-glucuronide[D]	22.10±0.32	16.75±1.20
Methylquercetin deoxyhexose glucuronide[E]	10.43±0.23	7.95±0.11
Quercetin 3-O-glucoside	1.41±0.06	0.53±0.01
Kaempferol 3-O-rutinoside	-	0.15±0.04
Ellagic acid	1.77±0.02	1.40±0.02
Methyl ellagic acid deoxyhexose[B]	1.47±0.00	0.54±0.02
Total ellagic acid derivatives	11.22±0.06[b]	5.78±0.27[c]
Total flavonols	72.02±0.40[a]	56.98±1.11[b]
Total flavan 3-ols	51.41±0.44[a]	35.83±0.52[b]
Total phenolic compounds	134.65±0.09[b]	98.59±0.85[c]

For the total compounds, in each row and for each sample (commercial or wild), different letters mean significant statistical differences between samples ($p<0.05$), where "[a]" and "[c]" correspond to the highest and lowest values, respectively.

Calibration curves used to quantify compounds which standards are not available: [A]-catechin, [B]-ellagic acid, [C]-gallic acid, [D]-quercetin-3-O-glucoside, [E]-quercetin-3-O-rutinoside, [F]-kaempherol-3-O-glucoside, [G]-kaempferol-3-O-rutinoside, [H]-p-coumaric acid.

- 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. Search and assessment methodology

Search terms: *Fragaria; Fragaria vesca, Fragaria moschata, Fragaria viridis, Fragaria x ananassa* strawberry leaves.

Databases: Pubmed, Embase, Medline, HealLink, Scopus, Toxnet. The search was performed between June 2015 and May 2017

Libraries: Department of Pharmacognosy and Pharmacology, Faculty of Pharmacy of the University Complutense of Madrid, Spain

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

There are no registered or authorised medicinal products in the EU/EEA Member States according to information provided by the national competent authorities upon inquiry in preparation of this Assessment Report. The overview is not exhaustive.

Overview of data obtained from non-marketed medicinal products

No data available.

Information on relevant combination medicinal products marketed in the EU/EEA

No data available.

Information on other products marketed in the EU/EEA (where relevant)

Strawberry leaves are included as part of several multi-components preparations marketed as food supplements in the EU, intended for the relief of non-specific acute diarrhea, as diuretic and 'to improve metabolism'.

2.1.2. Information on products on the market outside the EU/EEA

No information available.

2.2. Information on documented medicinal use and historical data from literature

Wild strawberry leaves are traditionally used as a herbal tea for the treatment of mild diarrhoea, as so called 'stomach tea and antiphlebitis tea' (Wichtl and Bisset 1994), and to promote urine flushing. Young leaves are used as a herbal tea in the food area.

In the folk medical practice, leaves are used in nephrolithiasis, gout, gall bladder stones and anemia. A decoction of the leaves of strawberries is popular as a diuretic. Tannins present in the leaves may exert an astringent effect on the gastrointestinal and oral mucosa and exert antibacterial activity.

Although literature references are mainly focused in *F. vesca* leaves, traditional use does refer to any of the *Fragaria* species included in this assessment report, as they are not differentiated when

collected. Moreover, the existing Austrian pharmacopoeia monograph includes the four species, either alone or as a mixture, and the existing literature considers them of similar medicinal value due to their comparable composition (Schönfelder and Schönfelder 2004).

The use of wild strawberry leaves as infusion or decoction has been mentioned for the treatment of diarrhoea, especially for children, for the inflammation of oral mucosa (as gargles) and in urinary-tract diseases, among others (Dragendorff 1898, Blaschek *et al*. 2006, Schneider 1974, van Wyk and Wink 2004, Wren 1975). For the treatment of diarrhoea and icterus, two tea-spoons per day of a decoction made with 375 g of young leaves in 1.15 l of water (until a final volume of 550 ml) is recommended. An infusion made with 4 g in 150 ml of water is recommended for diarrhoea in children. For the treatment of inflammation of the oral mucosa, some leaves are used to prepare a decoction for gargles (Blaschek *et al*. 2006). A standard dose of 1.0 g in 1 cup infusion, several times a day is described by Haffner *et al*. (2016).

Wild strawberries are widely used in the Balkans and especially Serbia, where numerous ethnobotanical data are published. In South-Western Serbia (Zlatibor district) *F. vesca* leaves as herbal tea (infusion) are used to treat diarrhea (Šavikin *et al*. 2013). In eastern Serbia, the wild leaves as herbal tea are used to treat cough (Zlatković *et al*. 2014). In Deliblato Sand, situated in the north of Serbia, the herbal tea (infusion) of *F. vesca* leaves is traditionally used in several indications: as antidiarrheal, antihelmintic, diuretic, blood purifier, relieves the pain from kidney stone and in liver and bladder area. The tea from very young leaves is used as antitussive, to 'heal' asthma, catarrh and cough. The tea from 'herbal parts' is used as nerve relaxant (Popović *et al*. 2014). The use of *Fragaria vesca* leaves was reported from the Kopaonik Mountains (Central Serbia) as diuretic, antigout (Jarić *et al*. 2007) laxative or for use in diarrhea and haemorrhoids in the Svrljiški Timok gorge in Eastern Serbia (Zlatković and Bogoslavljević 2014).

In Bulgaria, on the Eastern serpentine site of Rhodope Mountains, *Fragaria vesca* leaves are used as a herbal tea due to their supposed hypotensive, diuretic and anti-inflammatory effects (Nedelcheva *et al*. 2010). Leporatti and Ivancheva (2003) carried out a comparative study evaluating the therapeutic use of ethnobotanical plants in traditional folk medicine of Bulgaria and Italy. In both countries *Fragaria vesca, Fragaria viridis* and *Fragaria moschata* leaves have indications for use as a diuretic, astringent, anti-inflammatory and anti-atherosclerotic treatment. They are prepared as infusion or decoction. Moreover in Italy, it is traditionally used topically on the skin.

According to research of Tuttolomondo *et al*. (2014) in the Etna Regional Park, Eastern Sicily, *Fragaria vesca* leaves are traditionally used in cough, diarrhea, in mouth diseases, stomatic and throat diseases and wound treatment.

Ethnobotanical interviews were carried out over two years, from 2004 to 2006 in Western Navarra Pyrenees in Spain (Akerreta *et al*. 2007). A diuretic indication has been reported in the region, for use of the wild strawberry leaves.

In a study conducted in 2000 in the National Park "Serra de São Mamede" (Portugal), 45 informants participated to record the use of 150 plants in traditional medicine, including *Fragaria vesca* (Camejo-Rodrigues *et al*. 2003). The use of leaves of wild strawberry in the treatment of hypertension was recorded. Neves *et al*. (2009) performed ethnobotanical research in the region of Tras-os-Montes (northern of Portugal). They reported the use of *Fragaria vesca* leaves as diuretic, in diarrhoea and in gout.

Archival data on wild food plants used in Poland in 1948 published by Łuczaj (2008) show traditional use in Poland of the wild strawberry leaves as a herbal tea for diuretic effects.

Table 5 shows a summary of the traditional uses of strawberry leaves.

Table 5: Overview of historical data

Herbal preparations	**Documented use / Traditional use**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Reference**
Fragariae folium	As astringent in diarrhoea, jaundice, and mild diuretic	Oral hot infusion 1 tablespoon in 1 cup, 2 cups daily	Kosch 1939
Fragariae folium	Oral use as diuretic	Oral use 1 tablespoon of the leaves in 200 ml of water as *decoctum*. Drink 50 ml 2-4 times daily	Bobowska *et al*. 1977
Fragariae folium	Oral use as diuretic, astringent	Decoction 1-2 tablespoons for 1-2 glasses of water. Drink 2-3 times daily	Ożarowski *et al*. 1978
Fragaria vesca L. as herbal tea	1. Topical oral use in mouth and throat infection 2. Internal oral use as diuretic and in diarrhea	Decoction: 5 g of the leaves in 100 ml of water. Gargle the mouth and throat several times a day Infusion: 4 g in 100 ml of water. Drink 3-4 times a day a small cup	Wurzer 1994
Fragaria vesca Dried wild strawberry herb	Oral use: For supportive treatment of acute non-specific diarrhoea	Oral use: Herbal tea-pour 250 ml of boiling water over 2 tablespoons crushed strawberry leaf, steep for 10 minutes, and strain. Drink 3 cups daily	Podlech 1997

2.3 Overall conclusions on medicinal use

The evidence on the period of medicinal use for *Fragaria* leaves is limited to those references including all the species, with a traditional indication and posology (Table 6).

In the monograph, the posology of *Fragaria vesca* leaves as a diuretic in patients with mild urinary symptoms was calculated based on the publications of Bobowska *et al*. 1977 and Ożarowski *et al*. 1978.

Table 6: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Fragariae folium	As astringent in diarrhoea, jaundice, and mild diuretic	Oral hot infusion 1 tablespoon in 1 cup 2 cups daily	Kosch 1939
Fragariae folium	Oral use as diuretic	Oral use 1 tablespoon of the leaves as a decoction for 200 ml of water. Drink 50 ml 2-4 times daily	Bobowska *et al*. 1997
Fragariae folium	Oral use as diuretic, astringent	Decoction 1-2 tablespoons for 1-2 glasses of water. Drink 2-3 times daily	Ożarowski *et al*. 1978

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Antidiarrheal effect

Fragaria leaves due to their tannin content may have astringent effects. However, there are no experimental studies available to support this indication.

In folk medicine, the use of wild strawberry leaves is mentioned for mild diarrhoeas, especially in children, as well as for gargling for inflammations of the throat, oral mucosa and gums.

The use as antidiarrhoeic and to treat throat inflammations may be plausible due to the presence of tannins.

Diuretic effect

Although wild strawberry leaves are traditionally used to promote urine flow, the studies conducted to evaluate the diurectic activity are scarce and results are not conclusive.

In rats, the decoction of the leaves corresponding to a dose of 1 g drug/kg did not have any significant influence on diuresis following oral administration. The extract made from 0.5 g dried leaves with 20 ml ethanol 50% at a concentration of 100 µl/2.5 ml medium inhibited the proteolytic activity of the enzyme elastase by 85%; however, no data on the effect of a positive control are available (Blaschek *et al*. 2006).

3.1.2. Secondary Pharmacodynamics

3.1.2.1. Anti-inflammatory activity - in vitro *experiments*

Xantine oxidase inhibition

1) 20% aqueous ethanolic extract

2) 80% aqueous ethanolic extract

3) methylene chloride–methanolic extract

Havlik *et al* (2010) studied *in vitro* the xanthine oxidase inhibitory properties of 27 plants, *Fragaria vesca* included, traditionally used in Central-Eastern Europe for gout, arthritis or rheumatism treatment.

For this purpose three extracts were prepared from each 5 g powdered sample, including 20% aqueous ethanolic, 80% aqueous ethanolic and methylene chloride–methanolic extracts (50/50 CH_2Cl_2/MeOH) of *Fragaria vesca* leaves.

Extracts were tested on their inhibitory activity against xanthine oxidase *in vitro* at the starting concentration 200 µg/ml (Table 7), but the results did not show any evident effects.

Table 7: Xantine Oxidase inhibitory properties of selected medicinal plants (after Havlik *et al* 2010)

Plant	Extract	Inhibition (%) at 200 µg/ml	IC_{50} (µg/ml)	Polyphenol content (mg/g)
Fragaria vesca	20% EtOH	13.9±1.1	>200	78.9
	80% EtOH	53.6±4.2	170.5	102.3
	CH_2Cl_2/MeOH	56.0±0.3	179.7	114.3

[a] Gallic acid equivalent; –, no activity at 200 g/mL

Effects on cell viability, NO production, NO scavenging and on iNOS and COX-2 protein expression

Liberal *et al*. (2014) studied the antinflammatory activity of extracts from *Fragaria vesca* leaves:

Hydroalcoholic extract

The comminuted dried leaves were treated at room temperature with dichloromethane (1:10, w/v-2 times) and 50% aqueous ethanol (3 times) (1:10, w/v), filtrated and lyophilized. A yield of 30% expressed in dry plant was received. The extract was dissolved in phosphate buffered saline (PBS).

Effects of the **hydroalcoholic** extract of *Fragaria vesca* leaves on cell viability, NO production and NO scavenging activity were estimated in the cultured mouse leukemic monocyte macrophage cell line Raw 264.7.

NO production by the macrophages, cultured in the presence or in the absence of LPS, was evaluated after incubation with the extract at non-toxic concentrations of 80 µg/ml and 160 µg/ml. Control cells produced very low NO levels (0.35±0.23 µM), while the cells treated with LPS (for 24 hours) presented an increase of NO content (28.64±2.10 µM). In cells pretreated with the plant extract (160 µg/ml and 80 µg/ml), a significant decrease (40% and 31% respectively) of nitrite production was seen.

In another experiment, using S-nitroso-N-acetylpenicillamine (SNAP) as NO donor (300 µM), the mediums were collected after an incubation of 3 hours with SNAP and/or the **hydroalcoholic extract**

and nitrite concentration was measured by the Griess reaction. *Fragaria vesca* extract (only tested at 160 µg/ml) promoted a significant decrease of nitrite content (23% of inhibition) in the culture medium ($P<0.001$).

The authors tested further the effect of *Fragaria vesca* leaves **hydroalcoholic extract** on iNOS and COX-2 protein expression. By using the Western blot method, a significant iNOS increase in cells treated with LPS (299±30% of control) was found. Nevertheless, no differences were found between cells treated with LPS alone and cells treated simultaneously with the extract (no effect at 80 µg/ml or at 160 µg/ml). Likewise, while COX-2 activity was significantly induced upon stimulation by LPS, the pre-treatment with the extract (160 µg/ml only) did not inhibit LPS-induced COX-2 protein levels compared with LPS alone (1480±499% of control). Moreover, the *Fragaria vesca* leaves extract did not influence the activity of LPS on mRNA levels of iNOS and IL-1β in mouse macrophages. The authors concluded that inhibition of the nitrite production by the extract is probably the result of a 'direct nitric oxide scavenging'.

Effect on proteasome activity and autophagy

With reference to the crosstalk between ubiquitin-proteasome system (UPS) and autophagy, suggesting a synchronized function between these proteolytic systems, Liberal *et al*. (2014) studied the conversion of microtubule-associated protein light chain LC3-I to LC3-II, which has been used as a hallmark of autophagy. *Fragaria vesca* hydroalcoholic extract increased this marker of autophagy, i.e. the conversion of LC3-I into LC3-II. It suggests that the extract is activating autophagy.

Assessors comment:

Increasing evidence suggests that reduced autophagic activity may play a key role in the aging process. Since the reduction of autophagy processes progresses with age and is an expression of neurodegeneration, activation of this processess may have a beneficial therapeutic relevance.

Effect on prostaglandin biosynthesis and platelet activating factor (PAF) induced exytosis

Tunon *et al* (1995) studied anti-inflammatory effects of a number of medicinal plants, Fragariae vesca folium included. **Water extracts** were tested *in vitro* for inhibition of the biosynthesis of prostaglandins and platelet activating factor (PAF) induced exocytosis of elastase. An aqueous extract was prepared by extracting twice (1:20 and 1:10, 48 hours at room temperature), then the combined extracts freeze-dried and at the time of testing again dissolved in water. It was found that the *Fragaria* extract induced a prostaglandin synthesis inhibition of 15±2% (test concentration 0.2 mg/ml) and a PAF-exocytosis inhibition of 52±11% (test concentration 0.25 mg/ml). The authors classified an inhibition below 20% as insignificant, 20-40% as low, 40-70% as moderate and 70%-100% as high. The *Fragaria* extract was not among the most active of the 52 tested plants.

3.1.2.2. Anticoagulant activity - in vitro *experiments*

Several extract fractions were prepared from the dry leaves of *Fragaria vesca* (Pawlaczyk *et al*. 2009; 2013). In the procedure **methanol** and **acetone extraction** was performed resulting in isolation of 5 glycoconjugates: **Fv I**–**FvV**. These isolates were composed of carbohydrates and phenolic and protein constituents (Table 8).

Table 8: Characterisation of *F. vesca* Fv I–V glycoconjugates (after Pawlaczyk *et al*. 2013)

Plant conjugate	Yield (wt%)	[a]Total phenols [mM]	[b]Total sugar content (wt%)	Protein content (wt%)	[c]UA content (wt%)	Monosaccharide composition of carbohydrate part (wt%)							
						Rha	Fuc	Ara	Xyl	Man	Gal	Glc	[d]UA
Fv I	8.4	3.29	21.1	1.1	12.8	13.9	0.4	9.4	4.4	0.6	7.9	2.7	60.7
Fv II	5.3	1.17	28.5	0.5	8.8	13.2	n.d.	22.8	6.7	n.d.	15.4	11.0	30.9
Fv III	4.5	3.54	31.7	1.3	11.2	25.1	0.6	16.5	5.0	0.7	12.1	4.7	35.3
Fv IV	3.9	2.79	28.6	1.0	15.3	9.2	0.5	15.4	4.6	1.5	12.2	3.1	53.5
Fv V	3.7	0.81	29.0	1.8	12.9	3.9	n.d.	21.0	4.5	1.5	17.2	7.4	44.5

n.d. – not detected

[a]- Phenolic content expressed in mM of gallic acid equivalent (GAE) per 1 g of the plant glycoconjugate

[b]- Total sugar content determined by phenol-sulfuric assay

[c]- UA – total uronic acids content (wt%) in **Fv I–V** conjugates estimated by m-hydroxybiphenyl reagent

[d] - UA – uronic acid contents (wt%) calculated on carbohydrate parts in **Fv I–V** conjugates

Preparations **Fv I–V** were tested *in vitro* for their anticoagulant activity on human plasma. It has been found that the glycoconjugates are rich in hexuronic acids and phenolics, comparable to polysaccharide anticoagulants like glycosaminoglycans, i.e. heparin.

This anticoagulant activity of **Fv I–V** glycolconjugates was measured by activated partial thromboplastin time test (aPTT), prothrombin time test (PT), and thrombin time test (TT) in the human plasma, pooled from many healthy donors (Table 9, Table 10, Table 11). **Fv I–V** were tested in the range from 4000 µg/ml to 7.81 µg/ml.

Table 9: Activated thromboplastin time (aPTT) measurements of the *F. vesca* glycoconjugates *in vitro* in human pooled plasma. The bold value indicates that the clot was not observed in measured samples. Values are expressed as mean of 5 measurements±S.D. (Pawlaczyk *et al*. 2013)

Concentrations of a sample in the clotting mixture [µg/ml]	***In vitro* aPTT measurements [s]**				
	Fv I	**Fv II**	**Fv III**	**Fv IV**	**Fv V**
1000.00	**>600**	**>600**	**>600**	**>600**	**>600**
500.00	**>600**	528.9±6.1	**>600**	356.8±4.2	185.4±3.1
250.00	455.0±5.7	192.8±3.4	520.0±6.0	143.6±3.7	112.7±2.2
125.00	199.5±2.3	100.6±2.1	222.2±4.8	86.9±2.1	67.3±1.9
62.5	114.2±2.0	54.9±1.0	84.2±2.4	50.3±1.0	36.6±0.7
31.25	61.5±1.5	61.5±1.5	45.4±1.7	36.2±0.8	34.8±0.6
15.63	37.8±0.8	39.2±0.6	34.2±0.8	38.9±0.7	35.7±0.6
7.85	32.7±0.5	34.7±0.6	35.1±0.5	35.7±0.5	36.8±0.5
Control-0	36.8±0.6	36.8±0.6	36.8±0.6	36.8±0.6	36.8±0.6

Table 10: Prothrombin time (PT) measurements of the *F. vesca* glycoconjugates *in vitro* in human pooled plasma. The bold value indicates that the clot was not observed in measured samples. Values are expressed as mean of 5 measurements±S.D (Pawlaczyk *et al*. 2013).

Concentrations of a sample in the clotting mixture [µg/ml]	***In vitro* PT measurements [s]**				
	Fv I	**Fv II**	**Fv III**	**Fv IV**	**Fv V**
4000.00	**>300.0**	**>300.0**	**>300.0**	**>300.0**	43.6±1.8
2000.00	**>300.0**	144.7±6.2	**>300.0**	105.5±1.7	21.8±0.9
1000.00	92.8±3.9	48.0±2.8	**>300.0**	34.5±0.8	14.5±0.7
500.00	28.5±1.1	20.1±1.0	46.2±1.5	16.3±0.6	11.2±0.5
250.00	12.6±0.5	14.1±0.7	36.2±1.3	13.6±0.5	10.0±0.4
125.00	11.5±0.4	10.7±0.4	12.2±0.6	9.5±0.4	9.7±0.4

Concentrations of a sample in the clotting mixture [µg/ml]	***In vitro* PT measurements [s]**				
	Fv I	**Fv II**	**Fv III**	**Fv IV**	**Fv V**
62.50	10.9±0.4	10.2±0.3	9.9±0.5	10.5±0.5	9.1±0.3
31.25	9.4±0.3	9.9±0.3	10.5±0.4	10.8±0.4	9.6±0.4
15.63	9.8±0.4	10.1±0.3	11.9±0.5	10.6±0.4	11.7±0.4
7.81	10.5±0.3	10.2±0.2	10.3±0.4	11.0±0.3	11.2±0.2
Control–0	11.0±0.3	11.0±0.3	11.0±0.3	11.0±0.3	11.0±0.3

Table. 11: Thrombin time (TT) measurements, of the *F. vesca* glycoconjugates *in vitro* in human pooled plasma. The bold value indicates that the clot was not observed in measured samples. Values are expressed as mean of 3 measurements±S.D (Pawlaczyk *et al*. 2013).

Concentrations of a sample in the clotting mixture [µg/ml]	***In vitro* TT measurements [s]**				
	Fv I	**Fv II**	**Fv III**	**Fv IV**	**Fv V**
4000.00	**>300.00**	**>300.00**	**>300.00**	**>300.00**	**>300.00**
2000.00	**>300.00**	78.5±3.4	**>300.00**	71.6±3.3	38.1±1.7
1000.00	67.4±3.1	38.9±1.8	**>300.00**	22.4±1.0	27.6±1.2
500.00	22.1±0.9	34.5±1.1	36.4±1.0	18.7±0.8	22.1±0.9
250.00	20.8±0.8	28.6±0.9	24.6±0.8	17.0±0.7	22.6±0.9
125.00	17.7±0.6	17.7±0.7	20.1±0.8	13.5±0.6	18.9±0.7
62.50	12.5±0.4	14.6±0.6	14.9±0.6	11.7±0.5	14.9±0.6
31.25	12.6±0.5	12.1±0.4	12.8±0.5	12.0±0.4	13.2±0.5
15.63	12.1±0.4	12.3±0.5	11.9±0.5	12.3±0.5	12.0±0.4
7.81	12.7±0.6	11.5±0.4	12.1±0.4	12.0±0.4	12.5±0.4
Control–0	11.5±0.4	11.5±0.4	11.5±0.4	11.5±0.4	11.5±0.4

Presented *in vitro* results showed that all *Fragaria vesca* isolates displayed some anticoagulant activity, in the potency order Fv I > Fv III > Fv II > Fv IV > Fv V. It is apparent that only two glycoconjugates, i.e. Fv III and Fv I have been shown to have a significant activity, but still lower than that of unfractionated heparin. It turned out that the most active conjugates contained similar amounts of galacturonic acid, and the highest amount of phenolics.

3.1.2.3. Antithrombin and cytotoxic activity - in vitro *experiments*

Goun *et al*. (2002) determined the antithrombin activity of the **methylene chloride (a)** and **methanol (b) extracts** prepared from forty-five plants of Russia, wild strawberry leaves included. In

parallel, the cytotoxic activity of extracts was tested on mouse leukemia cells (L1210) (Table 12). The idea of the test was that the lower the activity of thrombin, the lower the coagulability, and therefore, the lower the possibility of tumor cells to adhere to any tissue or to spread. The authors postulated that simultaneous tests of antithrombin and cytotoxicity are suggested by the fact that 50% of patients with solid tumors have thrombosis. Furthermore, 95% of cancer patients show clotting activation.

The wild strawberry extract was prepared by extraction of the leaves (200 g dry weight) with methylene chloride (24 hours) and afterwards with methanol (24 hours) in a Soxhlet apparatus. The solvent was removed in vacuum to yield the extracts.

The authors summarised that eight plant extracts demonstrated activity of 90% or higher in the inhibition of thrombin (*Fragaria* not among them). Also, nine methanol extracts demonstrated activity of 90% or higher in the inhibition of L1210 (mouse leukemia) cell viability (*Fragaria* not among them). They admitted that 'even though, 23 methylene chloride extracts also have demonstrated activity of 95% or higher, future emphasis will be placed on the methanol extracts because they do not cause necrosis of the cells as is the case for methylene chloride extracts'.

Table 12: Antithrombin and cytotoxic activity of *Fragaria vesca* leaf extracts (Goun *et al*. 2002).

	Extract [a]	**Activity** [b]		**Extract** [a]	**Activity**[d]	
		A%	Type[C]		A%	Type[C]
***Fragaria vesca* leaves**	a	0	-	a	77	+ +
	b	0	-	b	11	0

[a] Type of extract: (a) methylene chloride extract; (b) methanol extract

[b] Antithrombin activity when compared with the blank solution: I% = (v_{sample}/v_{blank}) 100%

[c] + + +, 80/100% of activity; ++60-79% of activity; +30-59% of activity; 0, 1-29% of activity

[d] A cytotoxicity assay was used with L1210 as target cells and 10 µg per well concentration of the extract.

Both *Fragaria* extracts showed no antithrombin activity (test concentration not clear). The cytotoxicity tested at only one concentration (10 µg per well) was given with 77 % for the methylene chloride extract and 11% for the methanol extract. Results are questionable and practically irrelevant for the traditional use.

3.1.2.4. Cytotoxic activity - in vitro *experiments*

Fragaria vesca hydroalcoholic extract and derived fractions

The cytotoxic activity of an ellagitannin-enriched fraction (EEF) from *Fragaria vesca* leaves was studied in the cultured human hepatic carcinoma cell line HepG2 (ATCC HB-8065) by Liberal *et al* (2015). The hepatic cells viability after treatment with of both EEF and crude hydroalcoholic extract was determined and the half maximal inhibitory concentration (IC_{50}) was evaluated. It was found, that different concentrations of EEF during 24 hours exposure induced: IC_{50}=113 µg/ml; IC_{45}=80 µg/ml; IC_{40}=56 µg/ml; IC_{25}=23 µg/ml; IC_{15}=9 µg/ml.

EEF treated HepG2 cells were also analyzed via flow cytometry for alterations in the cell cycle, namely the distribution of G0/G1, S and G2/M phases. The proliferation of the cells was dose-dependently decreased. Higher concentrations of the EEF induced an increase in cells in the G2/M phase whereas lower doses promoted a significant increase in cells in G1 when compared to control cells.

In order to evaluate cell death and to discriminate between apoptosis and necrosis, cells were incubated with different concentrations of EEF for 24 h and then stained with annexin V-FITC/PI. The two higher concentrations of EEF promoted an increase in necrotic cells (annexin V-negative/PI-positive cells). This increase was accompanied by a rise, although not statistically significant, of annexin V-positive/PI-positive cells that correspond to late apoptotic/necrotic cells. Thus EEF induced features of necrosis and to some extent apoptosis in HepG2 cells.

It was furthermore observed that EEF promoted the accumulation of ubiquitinated proteins and in a dose-dependent way decreased chymotrypsin-like activity of the 26S proteasome after 6 and 24 hours of exposure.

In summary, *in vitro* laboratory studies provide some evidence on viability reduction of some cancer cells. Cytotoxic activity of strawberry leaves was observed in mouse L1210 leukemia cells (Goun *et al*. 2002), in the human promyelocytic HL_{60} cell line and its multidrug resistant sublines, exhibiting two different MDR phenotypes: HL_{60}/VINC (overexpressing P-glycoprotein) and HL_{60}/DOX (overexpressing MRP1 protein (Skupień *et al*. 2006) and in a human hepatic carcinoma cell line (HepG2–ATCC HB-8065) (Liberal *et al*. 2015).

3.1.2.5. Vasoactive effects – ex vivo *experiments*

The vasodilatory activity of the **aqueous wild strawberry leaves extract** was studied on endothelium-denuded and intact aortic rat rings exposed to nitric oxide (NO) synthase inhibitor L-NAME or to the cyclooxygenase inhibitor indomethacin (Mudnic *et al*. 2009). Dried leaves (15 g) were added into 150 ml of boiled deionised water and left at room temperature for 30 minutes without heating. The infusate was filtered and evaporated to dryness, yielding 1.44 g of wild strawberry extract. Afterwards the extract was redissolved to a final concentration of 6 g/100 ml. Isolated rat aortic rings (endothelium denuded and intact) were exposed to the following extract concentrations: 0.06, 0.6, 6 and 60 mg/100 ml. The rings were precontracted with test dose of noradrenaline (NA 10^{-7}mol/l). Subsequently endothelium dependent relaxation was induced by acetylcholine (ACh 10^{-6}mol/l). The functionality of endothelium was confirmed if 10^{-6}mol/l ACh induced more than 70% relaxation of precontracted rings. The relaxation of the rat aortic rings was expressed as the percentage decrease of NA-induced vasoconstriction. The maximum relaxation induced by the strawberry extract was 72.2±4.45. Removal of the endothelium caused a complete loss of vasodilatory response to the *Fragaria vesca* extract. Moreover, a strong inhibition of the vasodilatory response was seen after preincubation of the intact rings with NO synthase inhibitor, L-NAME, COX inhibitor–indomethacin. Nevertheless inhibitory activity of indomethacin was antagonised by the highest dose (60.0 mg/100 ml) of the extract. According to the authors, the results indicate, that the vasodilatory effect of the wild strawberry leaves aqueous extract is endothelium dependent. Nevertheless the inhibition of NO synthase activity seems more important than COX inhibition, because the latter could be antagonised by the highest experimental dose of the extract.

3.1.2.6. Cardiac effects - ex vivo *experiments*

The **aqueous extract of *Fragaria vesca* leaves** was tested in guinea pig isolated hearts (Mudnic *et al*. 2009). The wild strawberry extract was applied at concentrations of 0.06, 0.18, 0.6, and 1.8 mg/100 ml. Each concentration was perfused for 3.5 minutes with 15 minutes of washout periods. Heart contractility, electrophysiological activity, coronary flow and oxygen consumption were continuously monitored. The heart rate was not influenced by application of all consecutive doses of the extract. The initial control values for heart rate, AV conduction time and LVP were 224±6 beats per minute, 63±2 ms and 92±2 mm Hg, respectively. The initial control value of coronary flow was

13.1±0.5 ml per minute. The extract at concentrations of 0.06, 0.18, 0.6, and 1.8 mg/100 ml increased coronary flow by 3.6±1.2, 8.4±1.6, 32.7±5.0 and 44.5±4.5% over the control value, respectively. Oxygen extraction in the guinea pig hearts was significantly reduced by the consecutive doses of the extract as follows: by 3±1, 11±2, 27±2 and 34±4% from the control value of 78±2%.

3.1.2.7. Antimicrobial activity - in vitro *experiments*

Borah *et al.* (2012) evaluated the antimicrobial activity of four medicinal plants, including *Fragaria vesca* leaves. An **aqueous solution of a dry ethanolic extract prepared from dry plant material by percolation (ethanol 90%–no further detail)** was used to determine the diameter of the inhibition zones in the agar cultures discs of bacteria. Standard commercial discs of ciprofloxacin (5 µg/ml) were used as reference standard and ethanol (90%) impregnated discs were used as negative controls. The plates were incubated at 37°C for 24 hours in inverted position to estimate the antibacterial activity against selected bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* (Table 13).

Table 13: Inhibition zones in agar culture discs exposed to an aqueous solution of an ethanolic *Fragaria* extract (1 mg and 0.5 mg) against selected microorganisms compared to ciprofloxacin (5 µg) (after Borah *et al*. 2012)

Extracts / Positive control	***Fragaria vesca* extract**		**Ciprofloxacin**
Concentration	**1 mg per disc**	**0.5 mg per disc**	**5 µg per disc**
Microorganisms	Zones of inhibition (mm)		
Staphylococcus aureus	16.50±0.428	13.67±0.421	21.50±0.428
Escherichia coli	17.33±0.494	14.83±0.477	22±0.365
Pseudomonas aeruginosa	13.17±0.477	11.5±0.428	21±0.577

Marked antibacterial effect has been found for the *Fragaria* leaves ethanolic extract (0.5 mg and 1 mg samples) against *Staphylococcus aureus* and *Escherichia coli* strains. The *Pseudomonas aeruginosa* showed less growth inhibition to the *Fragaria* extract compared to the other bacterial strains.

Pereira *et al*. (2012) investigated leaf extract fractions on metallo-beta-lactamase, MBL VIM-2 producers *Pseudomonas aeruginosa* clinical strain isolates. Initially *Fragaria vesca* leaves were extracted with **ethanol (crude extract).** Afterwards **three fractions** were produced from that crude extract by elution with **50% aqueous methanol (Fa) 75% aqueous methanol (Fb), and 70% aqueous acetone (Fc)**. For the determination of MICs against MBL VIM-2 producers *Pseudomonas aeruginosa* standard microplate assays were used. The tested extracts gave MICs as follows: crude extract (MIC_{50}=10.35 mg/ml, MIC_{90}=20.7 mg/ml); fraction Fa (MIC_{50} and MIC_{90}=25 mg/ml); fraction Fb (MIC_{50}=6.25 mg/ml, MIC_{90}=12.5 mg/ml); fraction Fc (MIC_{50} and MIC_{90}=12.5 mg/ml). The most active antibacterial fraction was the Fb fraction consisting essentially of tannins: proanthocyanidins and ellagitannins.

Antimycobacterial effects of the **methanol extract** (no further details available) of *Fragaria vesca* leaves was studied by McCutcheon *et al.* 1997 (cited after Newton *et al*. 2000). It was found, that methanol extract induced of small zone of clearing of *Mycobacterium tuberculosis* at 50 µg extract per disc. No activity of the extract against *Mycobacterium avium* at 50 µg extract per disc has been established.

3.1.2.8. Antioxidant activity - in vitro *experiments*

Fragaria vesca water extract

The total antioxidant capacity of 70 plants, *Fragaria vesca* included, was estimated using the Ferric Reducing/Antioxidant Power (FRAP) assay as reported by Katalinic *et al* (2006). The total phenolic content of all infusions according to the Folin–Ciocalteu colorimetric method was in the range from 9 to 2218 mg/l. The FRAP range was determined at values of 0.06 to 25 mM/l. There was an overall significant linear correlation between total phenolic content and FRAP. *Fragaria vesca* infusions (leaves or herb not clear as indicated with 'herba folium') were prepared according to a standard protocol. To 3 g of plant material 200 ml of deionised water (98°C) were added. The infusion was left to stay at room temperature for 30 minutes. The extract was filtered and the liquid portion was analysed for its total phenol content and antioxidant capacity. Each sample was prepared in four repetitions (Table 14).

Table 14: The total phenolic content and related total antioxidant capacity determined as FRAP of *Fragaria vesca* infusate (Katalinic *et al*. 2006)

Plant material	Total phenolics (mg CE/l)[a]	FRAP (µmol/l)[b]	PAC[c]
Fragariae 'herba folium'	841	11022	3.8

[a] mg CE/l–miligram catechin equivalent per liter of infusate

[b] FRAP–ferric reducing/antioxidant power

[c] PAC-Phenol antioxidant coefficient, calculated as ratio FRAP

(lM/l)/total phenolics (lM CE/l)

The phenolic content and antioxidant properties of Fragariae 'herba folium' aquous extract, when measured with the FRAP assay were comparatively strong (11th strongest out of 70). The extract of *Fragaria vesca*, due to the relatively high value of the phenol antioxidant coefficient (PAC), can be considered a rich source of antioxidants.

Also another group (Buricova & Reblova, 2008; Buricova *et al*. 2011;) tested the antioxidant activity and phenolic content of some substances present in medicinal plants, wild strawberry leaves included. For the **water extract** preparation in the more specific 2011 study, the leaves of *Fragaria vesca* were ground and 1 g of the ground leaves was left in 50 ml of deionised water (98°C) for extraction during 20 minutes. Green tea preparations for comparison were prepared with shorter infusion time (2-4 min). Four methods were used: total phenolics assay (Folin-Ciocalteu method, TPC), ferric reducing antioxidant capacity (FRAP), oxygen radical absorbance capacity (ORAC) and free radical scavenging ability by the use of a stable DPPH radical. Additionally the determination of selected antioxidants was performed by HPLC (Table 15; Table 16).

Table 15: Antioxidant activity (DPPH, FRAP, ORAC) and total phenolic content (TPC) of *Fragaria vesca* water extract in comparison with green tea water extract (Buricova *et al*. 2011)

Medicinal plant	DPPH	FRAP	ORAC	TPC
Fragaria vesca	110.1±16.6	23.3±1.4	1062.0±143.9a	62.4±1.0
Green tea	175.2±20.9b	47.0±2.4	1628.6±62.8	84.8±1.0

Data are expressed as mean±SD (n=3, an=4, bn=6); DPPH in mg ascorbic acid/g of dry sample; FRAP in mmol $FeSO_4$/l; ORAC in µmol Trolox/g of dry sample; TPC in mg gallic acid/g of dry sample.

Table 16: Concentration of antioxidants in *Fragaria* leaves water extracts and their contribution (% AC) to the total antioxidant capacities of the extracts (Buricova *et al*. 2011)

Medicinal plant/compound	**c (mg/l)**	**% AC**	**c (mg/l)**	**% AC**	**c (mg/l)**	**% AC**
***Fragaria vesca* leaves**	sample I (2202±332)*		sample II (3295±357)*		sample III (2241±225)*	
Haklic acid	1.2	<1	5.9	<1	2.0	1
Ellagic acid	21.9±0.1	9.9	34.5±3.6	10.5	21.2±1.3	9.7
(+)-Catechin	45.6±2.4	10.1	29.8±12.2	4.4	98.8±9.9	21.6
Epigallocatechin	3.1	<1	4.1	<1	8.0±1.3	1.6
Procyanidin B1	3.0	<1	11.8±0.2	1.8	5.4±0.8	1.4
Total	>20.0		>16.7		>34.3	

The data are expressed as mean±SD (n=3 for compound with % AC>1); *Antioxidant capacities (DPPH) of leaves water extract are expressed as means±SD (n=3), mg ascorbic acid/l

The AC (radical scavenging capacities) (TPC, DPPH, ORAC, FRAP) of the studied water extract of the wild strawberry leaves were determined to be in the range of about 50% of the antioxidant capacity of green tea water extract. Furthermore, the AC (DPPH only) of herbal infusions were specifically compared to the capacities of other sources of. The AC of the *Fragaria* infusion studied was comparable to the AC of white wine, two times lower than the capacity of black tea infusion, three times lower than the capacity of green tea infusion and four times lower than that of red wine. Moreover it was reported that (+)-catechin, and ellagic acid significantly participated in the antioxidant activity of the water extract from wild strawberry leaves.

In the **ethanolic extract** of *F. vesca* leaves following phenolic compounds were identified: –(+)-catechin, (−)-epicatechin, ellagic acid, epigallocatechin gallate, hyperoside, isoquercitrin and three quercetin derivatives. Isolated compounds of Fragaria leaf extracts exhibited strong antioxidant activity. The range of total TEAC values summarised from all single identified and unidentified compounds was 191.23–609.36 mol/g and 178.63–642.20 mol/g of ABTS and FRAP, respectively (Raudonis *et al*. 2012).

Table 17: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Fragaria vesca, folium 1) aqueous ethanolic extract 2) 80% aqueous ethanolic extract 3) methylene chloride–methanolic extracts	Starting concentration 200 µg/m	Extracts were tested on their inhibitory activity against xanthine oxidase *in vitro*	Havlik *et al*. 2010	The results showed no significant inhibitition of the enzyme.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Hydroalcoholic extract of *Fragaria vesca* leaves.	80 and 160 mg/ml	*In vitro* Effect on cell viability, NO production and NO scavenging activity	Liberal *et al*. 2014	The pre-treatment with the extract did not inhibit LPS-induced COX-2 protein levels compared with LPS alone (1480±499% of control). For non-cytotoxic concentrations (80 and 160 mg/ml) the extract inhibited nitrite production, probably due to a direct nitric oxide scavenging.
Five glycoconjugates composed of carbohydrates and phenolic and protein constituents: Fv I–FvV *(*extract fractions from *Fragaria vesca* leaves methanol and acetone extraction)	Concentrations of a sample in the clotting mixture tested in the range from 4000 to 7.81 µg/ml	*In vitro* Anticoagulant activity in human plasma measured by activated partial thromboplastin time test , prothrombin time test, and thrombin time test	Pawlaczyk *et al*. 2009; 2013	*Fragaria vesca* isolates displayed anticoagulant activity, in the potency order Fv I>Fv III>Fv II>Fv IV>Fv V. Only Fv III and Fv I have shown significant activity, but lower than that of unfractionated heparin.
Fragaria vesca methylene chloride extract *Fragaria vesca* methanol extract	50 µl of plant extracts was used	*In vitro* Antithrombin bioassay was performed according the method of Medeiros *et al*. (2000). Cytotoxic activity was tested in mouse leukemia cells (L1210)	Goun *et al*. 2002	No antithrombin activity of *Fragaria vesca* extracts. Cytotoxic activity was evaluated as 'medium' for the methylene chloride extract (77% inhibition compared to the control) and as insignificant for the methanol extract (11% inhibition compared to the control)

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Fragaria vesca leaves aqueous extract	Following concentrations of the extract were used: 0.06, 0.6, 6 and 60 mg/100 ml.	*Ex vivo* Vasoactive effects. Vasodilatory activity was studied on endothelium-denuded and intact aortic rat rings exposed to nitric oxide (NO) synthase inhibitor L-NAME or cyclooxygenase inhibitor indomethacin	Mudnic *et al*. 2009.	Maximum relaxation induced by the strawberry extract was 72.2±4.45. Removal of the endothelium caused a complete loss of vasodilatory response to the *Fragaria vesca* extract. The inhibitory activity of indomethacin was antagonised by the highest dose (60.0 mg/100 ml) of the extract. Results indicate that the vasodilatory effect of the wild strawberry leaves aqueous extract is endothelium dependant.
Fragaria vesca leaves aqueous extract	Concentrations of 0.06, 0.18, 0.6, and 1.8 mg of the extract per 100 ml	*Ex vivo* Cardiac effects Guinea pig isolated hearts	Mudnic *et al*. 2009	Concentrations of the extract of 0.06, 0.18, 0.6, and 1.8 mg/100 ml increased coronary flow by 3.6±1.2, 8.4±1.6, 32.7±5.0 and 44.5±4.5% over the control value, respectively. Oxygen extraction in the guinea pig hearts was significantly reduced by the consecutive doses of the extract as follows: by 3±1, 11±2, 27±2 and 34±4% from the control value of 78±2%.
Fragaria vesca leaves ethanolic extract	1 mg of the extract per disc; 0.5 mg of the extract per disc	*In vitro* Antimicrobial activity Estimation of the antibacterial activity against *Staphylococcus aureus*,	Borah *et al*. 2012	Substantial antibacterial effect has been found for the *Fragaria* leaves ethanolic extract (0.5 mg and 1 mg samples) against *Staphylococcus aureus* and *Escherichia coli* strains.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
		Escherichia coli, *Pseudomonas aeruginosa*		*Pseudomonas aeruginosa* showed less growth inhibition
1. *Fragaria vesca* ethanol crude extract 2. 50% aqueous methanol (Fa) extract 3. 75% aqueous methanol (Fb) extract 4. 70% aqueous acetone (Fc) extract	MIC's range: 6.25 mg/ml-25 mg/ml	*In vitro* Estimation of the antibacterial activity against Pseudomonas aeruginosa	Pereira *et al*. 2012	crude extract: MIC_{50}=10.35 mg/ml, MIC_{90}=20.7 mg/ml; fraction Fa: MIC_{50} and MIC_{90}=25 mg/ml; fraction Fb: MIC_{50}=6.25 mg/ml, MIC_{90}=12.5 mg/ml; fraction Fc: MIC_{50} and MIC_{90}=12.5 mg/ml. The most active fraction was Fb consisting essentially of proanthocyanidins and ellagitannins.
Fragaria vesca leaves methanol extract	50 µg extract per disc	*In vitro* Antimyco-bacterial effects	McCutcheon *et al.* 1997 (cited after Newton *et al*. 2000).	Methanol extract induced of small zone of clearing. No activity of the extract against *Mycobacterium avium* at 50 µg extract per disc has been established.

3.1.3. Pharmacodynamic interactions

No data available.

3.1.4. Conclusions

The leaves from *Fragaria vesca* L.*, Fragaria moschata* Weston*, Fragaria viridis* Weston*, Fragaria x ananassa* (Weston) Duchesne ex Rozier, are traditionally used as herbal tea in European traditional medicine as diuretics and to treat diarrhoea.

The scientific information available on the pharmacological activity of *Fragraria* leaves is limited. One study was performed with a dosage of 1 g/kg in rats (oral administration) but showed no significant influence in diuresis. However, some reported pharmacological effects are partially consistent with the traditional use.

The astringent effect of strawberry may be due to the high tannins content (at least 3% refererred to dried drug), although no pharmacology studies proving this activity have been published.

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

No data available.

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

3.3.1. Single dose toxicity

Herbal preparations

There are no data available on the acute toxicity of *Fragaria* leaves.

3.3.2. Repeated dose toxicity

Subacute, Chronic Toxicity

There are no data available.

3.3.3. Genotoxicity

Mutagenicity

There are no data available.

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

No data available.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

No toxicity studies are available for *Fragaria* leaves.

Adequate tests on toxicity, genotoxicity and carcinogenicity have not been performed.

Nontheless, according to Gardner and McGuffin (2013), *F. vesca* can be considered as safe (Class 1) and no clinically-relevant interactions are expected (Class A).

3.4. Overall conclusions on non-clinical data

The scientific information available on the pharmacological activity of *Fragraria* leaves is limited. The relatively high content of polyphenols such as elagitanins, pedunculagin, gallic and chlorogenic acids and procyanidins partially explains the usage of the wild strawberry leaves. Results from relevant non-clinical experimental studies are scant, but the astringent, anti-inflammatory, antimicrobial and antiadhesive properties of the tannins present in the herbal substance can explain the traditional uses of the wild strawberry leaves for symptomatic treatment of mild diarrhoea.

There is no non-clinical information on the safety of *Fragaria* leaves. As there is no valid information on reproductive and developmental toxicity the use during pregnancy and lactation cannot be recommended.

Tests on genotoxicity and carcinogenicity have not been performed.

4. Clinical Data

4.1. Clinical pharmacology

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

No data available.

Clinical Studies

No data available.

4.2. Clinical Efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

No data available.

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

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

No data available from clinical trials.

5. Clinical Safety/Pharmacovigilance

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

There are no data available with respect to adverse effects related to *Fragaria* leaves or corresponding preparations use (Blaschek *et al*. 2006). According to Gardner and McGuffin (2013), strawberry leaves can be safely consumed when used appropriately.

Moreover, there are no restrictions to its use as an admixture (flavouring agent) in herbal teas (Bundesanzeiger 1990, DeSmet *et al*. 1993; Grattan and Harman 1985; Rossoff *et al*. 2002; Van Wyk and Wink 2004).

According to Rossoff *et al*. (2002), and due to their salicylates content, the only expected adverse effect is allergic contact urticaria.

5.2. Patient exposure

No data available.

Aside from market presence in mixed herbal teas and data from preclinical studies, there are no specific data concerning patient exposure.

5.3. Laboratory findings

No data available.

5.4. Safety in special populations and situations

5.4.1. Use in children and adolescents

Particular use in children has not been reported. Therefore, the use in children up to 12 years is not recommended.

5.4.2. Contraindications

Conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal disease).

Hypersensitivity to strawberry is reported. In the *Rosaceae* family allergic reactions mediated by cross-reactive phenomena have been identified linked to birch pollinosis (Zuidmeer *et al*. 2006; Eriksson 2004). Some allergene gene families were established for *Fragaria vesca* L., which include: PR-10, nsLTP and profilin (Hyun and Kim 2011).

5.4.3. Special Warnings and precautions for use

There are no adverse effects reported derived from the use of strawberry leaves.

To ensure an increase of the amount of urine, adequate fluid intake is required during treatment.

If complaints or symptoms such as fever, dysuria, spasms or blood in urine occur during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

If recurrent diarrhoea or bloody stools occur, a doctor or a qualified health care practitioner should be consulted.

5.4.4. Drug interactions and other forms of interaction

No data available.

5.4.5. Fertility, pregnancy and lactation

No data available.

5.4.6. Overdose

No cases of overdose have been reported.

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

No data available.

5.4.8. Safety in other special situations

Not applicable

5.5. Overall conclusions on clinical safety

On the basis of the information on traditional use, comminuted *Fragaria* leaves, are not considered harmful in the specified condition of use.

Wild strawberry leaves should be used with caution in persons with allergy to strawberry fruit (Wichtl and Bisset 1994).

6. Overall conclusions

Well-established use cannot be accepted for *Fragaria vesca* L., *Fragaria moschata* Weston*, Fragaria viridis* Weston*, Fragaria x ananassa* (Weston) Duchesne ex Rozier, folium, due to the absence of medicinal products in the European Union and the lack of data to recognise efficacy.

Traditional medicinal use of *Fragaria vesca* L., *Fragaria moschata* Weston*, Fragaria viridis* Weston*, Fragaria x ananassa* (Weston) Duchesne ex Rozier, folium, is documented in several handbooks throughout a period of at least 30 years (15 years in the European Community). The comminuted herbal substance from *Fragaria* can be considered as safe when used in recommended dosages under the conditions specified in the monograph.

The comminuted herbal substance of *Fragaria vesca*, *F. moschata*, *F. viridis* and *Fragaria x ananassa* leaves for aqueous preparations (infusions and decoctions) is presented in source handbooks. The main indications with some information on posology are the increase in the amount of urine and to treat mild or acute diarrhea. However, these indications are not supported by preclinical or clinical studies. Relevant information regarding preparation and dosage has been included in the monograph.

Long-standing traditional medicinal use of the wild strawberry leaves within the European Union for at least 30 years according to Directive 2004/24/EC is considered fulfilled for the comminuted herbal substance and indications:

1) Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

This wording is in compliance with comparable substance monographs (e.g. monographs on *Equisetum arvense* L., herba, *Taraxacum officinale* Weber ex Wigg, radix cum herba, *Betula pendula* Roth, folium; *Orthosiphon stamineus* Benth., folium).

2) Traditional herbal medicinal product used for the symptomatic treatment of mild diarrhea.

Wild strawberry leaves cannot be recommended for oral use in children up to 12 years of age due to lack of sufficient safety data.

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended. No fertility data is available.

Due to the lack of data on genotoxicity, carcinogenicity, reproductive and developmental toxicity, a European Union list entry for *Fragaria vesca*, *Fragaria moschata, Fragaria viridis* and *Fragaria x ananassa* cannot be recommended.

Annex

List of references