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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

Draft

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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Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Fragaria vesca* L., *Fragaria viridis* West., *Fragaria moschata* West.,*Fragaria x ananassa* (West.) Duchesne ex Rozier, folium. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on 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 monograph.

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1. Introduction

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

Herbal substance

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 wild 20 recognized 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 the form of the so-called "semperflorens or alpine" (Darrow 1966). The cultivated strawberry originated in France around 1750 from a random hybridization 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 hybridization 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 sure 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 the self-collected leaves of several species of Fragaria in various parts of Austria was 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 Ph (Österreichisches Arzneibuch 2013, 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 (C6H603; 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 leaves.

Common wild strawberry is a perennial plant of the family Rosaceae native to Europe and northern Asia which grows in meadows, woods and along roadsides (Mabberley 2002). The plant is native from the west of Ural mountains by northern Europe and across the North American continent. The present Fragaria taxonomy includes 20 named wild species which are distributed in the north temperate and Holarctic zones (Hummer et al. 2011). It is a perennial plant a height of about 5 -20. Leaves are complex (three-lobed, feathery) and have serrated edges and hairy petioles. Wild strawberry produces stolons and is flourishing and bearings fruits 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. It grows in woods, forest glades and wood margins and it is a light demanding species (Labokas and Bagdonaite 2005).

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, 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 salicylic acid, cinnamic acid, caffeic acid and chloro-genic acid. They also contain quercetin and quercitrin, 2.2% rutin as well as catechin and el-lagitannins, including pedunculagin, together with 5-11% condensed tannins (oligomeric proanthocyanides) (ARS 2016a, Blaschek et al. 2006, 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 gallic acid, cinnamic acid, caffeic acid and chlorogenic acid and 2.3% rutin, together with tannins (Blaschek et al. 2006).

Flavonoids

Haghi and Hatami (2010) studied the content of flavonoids in the wild strawberry leaves and found mean content of analytes for quercetin - 2.16 ± 0.0 and for kaempferol 0.34 ± 0.02 miligrams per gram of dry samples of the Fragaria leaves. Najda and Dyduch (2009a), Dyduch and Najda (2009) has found, that cultivated species of the wild strawberry leaves (*Fragaria vesca* “Regina”) contained more flavonoids (4%) as compared to herb collected from natural habitats (3%). It has been found out in Western Carpathians mountains, that *Fragaria vesca* occurs in a variety of habitats with different measured parameters (number of leaves, length of the longest leaf, dry weight and total flavonoid content). The flavonoid contents in the leaves were as a rule decreasing from lower to higher altitudinal zones (Pearson correlation coefficient R=0.770) (Malinikova et al. 2013).

Phenolic acids

Haghi and Hatami (2010) studied the content of phenolic acids in the wild strawberry leaves and found mean content of analytes for an unhydrolyzed ellagic acid – 1.72 and for hydrolysed ellagic acid – 21.66 miligrams per gram of dry samples of the Fragaria leaves.

In the methanolic extract of *Fragaria vesca* after field-grown leaves-direct extraction (Yildrim and Turker 2014) presence of such 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 cultivated species of the wild strawberry leaves (*Fragaria vesca* "Regina") contained less of phenolic acids which was higher in the wild forms with variation from 1.3% to 1.8% Ivanov *et al* (2014) found, that the most convenient method for extraction of procyanidins (B1, B2 and B5) from *Fragaria vesca* leaves was the method of using of 56% acetone-water solent system with time of ultrasonic extraction 50 min with frequency 35 kHz . As the resut they obtained the maximum amount of procyanidins 124.0 mg/100 g dry biomass.

Elagitannins

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

Both thin layer chromatography (TLC) and liquid chromatography (LC) methods were used for 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]				
Herbal medicine	**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

In the Fragaria vesca leaves terpene (R)-linalool was determined at high enantiomeric purity (ee >90%) by Hampel et al. (2006) by GC-MS analysis. The chromatographic analyses (GC-MS) 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-dry leaves contained from 0.46% ("Baron von Solemacher") to 0.62% ("Rugia) of essential oils. 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

On the way of metabolic degradation of aromatic compounds and phenolics, protocatechuic or gallic acid is formed and is 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, Duch. 239 mg/100 g which corresponds to the content as such in the Rosa Damascena Mill. = 235 mg/100 g of the plant material.

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

Phytochemical characterization of *Fragaria vesca* leaves was studied by Liberal *et al*. (2012). HPLC-PDA phenolic profiles were recorded at 280 nm. The hydroalcoholic extract evidenced the presence of proanthocyanidins, flavonols, and ellagic acid and its derivatives. Twenty 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]
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)	nd
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	nd
Citric acid	1.08 ± 0.06[c]
Fumaric acid	nd
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 "d" correspond to the highest and lowest values, respectively.

Table 4. Phenolic compounds quantification/estimation (mg/g) in the 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
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.

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

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

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 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

sppontaneusly. Moreover, the existing pharmacopoeia monograph includes the four species, both isolated or as a mixture, and the existing literature considers them of same medicinal value due to their comparable composition (Schönfelder and Schönfelder 2004).

The use of wild 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 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, a decoction made with 375g of young leaves in 1.15 L of water until a final volume of 550ml is recommended; to take two tea-spoons per day. An infusion made with 4g in 150ml of water is recommended for diarrhoea in children. For the treatment of inflammation oral mucosa, some leaves are used to prepare a decoction for gargles (Valnet 1983; 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 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. Tea from very young leaves is used as antitussive, for healing asthma, catarrh and cough. Tea from herbal parts is used as nerve relaxant (Popović et al. 2014). The use of *Fragaria vesca* leaves was registered from the Kopaonik Mountains (Central Serbia) as diuretic, antigout (Jarić et al. 2007) laxative or for use in diarrhea and haemorrhoids in in the Svrljiški Timok gorge in Eastern Serbia (Zlatković and Bogoslavljević 2014).

In the Bulgaria on the Eastern serpentine site of Rhodope Mountains Fragaria vesca leaves are used as a herbal tea due to their 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 in infusion or decoction forms. On the other hand, in Italy the traditional application is limited to use as astringent and topical application on the skin.

According to research of Tuttulomondo et al. (2014) in the Etna Regional Park, Eastern Sicily, Italy *Fragaria vesca* leaves are traditionally used in cough, diarrhea, in mouth diseases, stomatic and throat diseases and wounds 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 in establishment of the use 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 registered. Neves et al. (2009) performed ethnobotanical research in the region of Tras-os-Montes (northern of Portugal). They found the use of *Fragaria vesca* leaves as diuretic, in the 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 (where relevant) Posology Duration of use	Reference
Folia Fragariae	As astringent in diarrhoea, jaundice, and mild diuretic.	Oral hot infusion 1 tablespoon on 1 cup, 2 cups daily.	Kosch 1939
Fragariae leaves	Oral use as diuretic	Oral use 1 tablespoon of the leaves as decoctum for 200 ml of water. Drink 50 ml 2-4 times daily	Bobowska et al. 1977
Fragaria leaves	Oral use as diuretic, astringent	Decoction. 1-2 tbsp for 1–2 glasses of water. Drink 2 – 3 times daily	Ożarowski et al. 1978
Fragaria vesca *L*	1.Topical oral use in mouth and throat infection 3. Internal oral use as diuretic and in diarrhea	1) Decoction: 5g of the leaves in 100 ml of water. Gargle the mouth and throat several times a day. 2) Infusion : 4g 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 tbsp 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 restricted to those references including all the species, with a traditional indication and posology (Table 6).

In the monograph, dosing of Fragaria vesca leaves as a diuretic in patients with mild urinary symptoms were 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	Strength Posology	Period of medicinal use
Folia Fragariae	As astringent in diarrhoea, jaundice, and mild diuretic.	Oral hot infusion 1 tablespoon on l cup, 2 cups daily.	Kosch 1939
Fragariae leaves	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. 1977
Fragaria leaves	Oral use as diuretic, astringent	Decoction. 1-2 tbsp 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 are strongly astringent probably due to their tannin content. 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, there no data on the effect of a positive control are available (Blaschek et al. 2006).

3.1.2. Secondary Pharmacodynamics

Anti-inflammatory activity

Xantine oxidase inhibitory properties

***In vitro* Experiments**

***Fragaria vesca* folium**

1) aqueous ethanolic extract
2) 80% aqueous ethanolic extract
3) methylene chloride-methanolic extracts

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

For this purpose three extracts were prepared from each 5g powdered sample, including 20% aqueous ethanolic, 80% aqueous ethanolic and methylene chloride-methanolic extracts (50/50 CH2Cl2/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 evident effects.

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

Plant	Extract	Inhibition (%) at 200 μg/mL	IC50 (μg/mL)	Polyphenol content (mg/g)[a]
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

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

1. The **hydroalcoholic** extract of *Fragaria vesca* leaves.

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).

2. The **aqueous extract** of Fragaria vesca leaves.

The dried and comminuted leaves were inserted to the boiled water (4g:150mL, w/v) and left in room temperature for 15 min and it was filtrated. Afterwards the infusion was lyophilized yielding of23% of dried plant.

***In vitro* Experiments.**

*Effect of Fragaria vesca leaves **hydroalcoholic extract** on cell viability, NO production and NO scavenging activity*

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

Concentrations of 80 μg/mL and 160 μg/mL of the extract was evaluated on NO production by macrophages, cultured in the presence or in the absence of LPS,

Control cells produced very low NO levels (0.35 ± 0.23 µM), while the cells treated with LPS (for24h) 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 of nitrite production was seen. Using S-nitroso-N-acetylpenicillamine (SNAP) as NO donor (300 µM). The mediums were collected after an incubation of 3h with SNAP and/or the extract and nitrite concentration was measured by the Griess reaction. *Fragaria vesca* extract (160 µg/mL) promoted a significant decrease of nitrite content (23% of inhibition) in the culture medium ($P<0.001$).

*Effect of Fragaria vesca leaves **hydroalcoholic extract** on iNOS and COX-2 protein expression.*

By using Western blot method, a significant iNOS increase in cells treated with LPS (299 ± 30% of control) was found.

Nevertheless there no differences were found between cells treated with LPS alone and cells treated simultaneously with the extract (both concentrations).

COX-2 activity was also significantly induced upon stimulation by LPS; however the pre-treatment with the extract (160 µg/mL) 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. Inhibition of the nitrite production by the extract is probably the result of a direct nitric oxide scavenging.

*Effect of Fragaria vesca leaves **hydroalcoholic extract** on NF-κB pathway*

Fragaria vesca extract increased a marker of autophagy, the conversion of microtubule-associated protein light chain LC3-I into LC3-II. This finding definitely suggests that the extract is activating autophagy. Moreover the inhibition of proteasome activity was noted resulting in to an accumulation of the ubiquitin.

Tunon et al (1995) studied anti-inflammatory effects of a number of medicinal plants, *Fragariae vesca* folium included. Anti-inflammatory effects of **water extract** were tested *in vitro* for inhibition of the biosynthesis of prostaglandins and platelet activating factor (PAF) induced exocytosis of elastase. Aqueous extract was prepared twice (1:20 and 1:10) and then freeze-dried and at the time of pharmacological testing again dissolved in water. Experiments with lyophilized water extracts started at a concentration of 1mg / ml. It was found that the extract induced prostaglandin synthesis inhibition in 15 ± 2% and PAF-exocytosis inhibition at 52 ± to 11%.

Anticoagulant activity

In vitro Experiments

Fragaria vesca leaves methanol extract
Fragaria vesca leaves acetone extract

Several extracts were prepared from the dry leaves of *Fragaria vesca* (Pawlaczyk *et al*. 2009; 2013). In the procedure **methanol** and a**cetone** e**xtraction** 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. Characterization 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%)	cUA 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 as all tested glycoconjugates are rich in hexuronic acids and phenolics, similar to polysaccharide anticoagulants like glycosaminoglycans, i.e. heparin.

This anticoagulant activity of **Fv I–V** glycoconjugates 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).

The plant glycoconjugates **Fv I–V** were tested in the range of concentration from 4000 to 7.81 µg/mL, in order to evaluate the strength of the biological activity.

Table 9.Activated thromboplastin time (aPTT) measurements of the *F. vesca* glycoconjugates made in vitro experiments 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 made in vitro experiments 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**	
2000.00	**>300.0**	144.7±6.2	**>300.0**	105.5±1.7	
1000.00	92.8±3.9	48.0±2.8	**>300.0**	34.5±0.8	
500.00	28.5±1.1	20.1±1.0	46.2±1.5	16.3±0.6	
250.00	12.6±0.5	14.1±0.7	36.2±1.3	13.6±0.5	
125.00	11.5±0.4	10.7±0.4	12.2±0.6	9.5±0.4	
62.50	10.9±0.4	10.2±0.3	9.9±0.5	10.5±0.5	
31.25	9.4±0.3	9.9±0.3	10.5±0.4	10.8±0.4	
15.63	9.8±0.4	10.1±0.3	11.9±0.5	10.6±0.4	
7.81	10.5±0.3	10.2±0.2	10.3±0.4	11.0±0.3	
Control – 0	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 made in vitro experiments 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 tests showed that all *Fragaria vesca* isolates displayed the 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 biological activity, but lower than that of unfractionated heparin.

It turned out that the most active conjugates contain similar amounts of galacturonic acid, and the highest amount of phenolics.

Anticancer and antithrombin activity

In vitro Experiments

Fragaria vesca leaves methylene chloride extract
Fragaria vesca leaves methanol extract

Goun et al. (2002) determined the antithrombin activity of the m**ethylene chloride (**a) and **methanol** (b) **extracts** prepared from forty-five plants of Russia, wild strawberry leaves included. The idea of the test was, that the lower the activity of thrombin, the lower the coagulability, and therefore, the lower the possibility tumor cells have of adhering to any tissue or of spreading.

Anticancer activity of the tested extracts were tested on mouse leukemia cells (L1210). (Table 12).

The idea of simultaneous tests of antithrombin and anticancer is proposed due to condition when 50% of patients with solid tumors have thrombosis. Moreover, furthermore, 95% of cancer patients show clotting activation. The wild strawberry extract was prepared by extraction with methylene chloride and afterwards with methanol. The leaves (200 g dry weight) were extracted in sequence with methylene chloride (24 h) and ethanol (24 h) in a Soxhlet apparatus. The solvent was removed in vacuum to yield the methylene chloride extract and then the methanol extract.

Table 12. Antithrombin and anticancer activity of some Russian plants (Goun *et al*. 2002).

Plant species (family	Extract[a]	Activity [b]		Extract	Activity [d]	
		A%	Type [C]		A%	Type [C]
Fragaria vesca leaves	a	0	-	a	77	+ +
	b	0	-	b	11	0

[a] Indication of type of extract as follows: (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 mg per well concentration of the extract.

Cytotoxic activity

In vitro Experiments

Fragaria vesca hydroalcoholic extract

Cytotoxic activity of an ellagitannin-enriched fraction (EEF) from *Fragaria vesca* leaves was studied on the culture of the human hepatic carcinoma cell line (HepG2 – ATCC HB-8065) by Liberal et al (2015). Moreover, the hepatic cells viability after treatment with of both EEF and crude hydroalcoholic extract was determined and the half maximal inhibitory concentration (IC50) was evaluated. It was found, that different concentrations of EEF during 24 h exposure induced: IC50= 113 µg/mL; IC45 =80 µg/mL; IC40= 56 µg/mL; IC25= 23 µg/mL; IC15= 9 µg/mL. Afterwards EEF were analyzed for the distribution of G0/G1, S and G2/M phases.The EEF produced cel arrest at G2/M checkpoint. The proliferation of the cells was dose-dependently decreased. It was also found, that ellagitannin-enriched fraction induced after 24 h treatment both features of necrosis and apoptosis in HepG2 cells. This fraction also promoted the accumulation of ubiquitinated proteins and in a dose-dependent way decreased chymotrypsin-like activity of the 26S proteasome after 6 and 24 h of exposure.
Laboratory studies provide evidence on possible reduction of certain cancers as anticancer activity of strawberry leaves was observed on a model of L1210 leukemia target cells (Goun et al. 2002), promyelocytic HL60 cell line and its multidrug resistant sublines, exhibiting two different MDR phenotypes: HL60/VINC (overexpressing P-glycoprotein) and HL60/DOX (overexpressing MRP1 protein (Skupień et al. 2006) and on human hepatic carcinoma cell line (HepG2 – ATCC HB-8065) (Liberal et al. 2015).

Vasoactive effects

***Fragaria vesca* leaves water extract**

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 cyclooxygenase inhibitor indomethacin were used (Mudnic *et al*. 2009). Dried plant leaves (15 g) was added into 150 ml of boiled deionised water and left at room temperature for 30 min without heating. The infusates were filtered and evaporated to dryness, yielding 1.44 g of wild strawberry extracts. Afterwards the extract was redissolved to final concentration of 6 g/100 ml. Isolated rat aortic rings (endothelium denuded and intact) were exposed to the following doses of the extract: 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 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. Maximal 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 antagonized by the highest dose (60.0 mg/100 ml) of the extract. Received 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 antagonized by the highest experimental dose of the extract.

Cardiac effects

***In vitro* experiments**

***Fragaria vesca* leaves water extract**

The aqueous extract of *Fragaria vesca* leaves was estimated on guinea pig isolated hearts (Mudnic *et al*. 2009). In the isolated hearts, 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 min with 15 min 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/min, 63 ± 2ms and 92 ± 2mm Hg, respectively. The initial control value of coronary flow was 13.1 ± 0.5 ml/min. 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%.

Antimicrobial activity
***In vitro* experiments**

***Fragaria vesca* leaves water extract**
***Fragaria vesca* leaves methanol extract**
Borah *et al.*(2012) evaluated the antimicrobial activity of the medicinal plants, including *Fragaria vesca* leaves. In local tradition wild strawberry leaves preparation has been used in the treatment of rheumatism, gout diarrhea, digestive upsets and to stimulate the appetite.

To the *in vitro* studies, an aqueous solution of the dry ethanolic extract prepared from the dry plant material by percolation method (ethanol 90% – no further detail) was used to determine the diameter of the inhibition zones in the agar cultures discs of bacteria. A standard commercial discs of ciprofloxacin (5µg/ml) were used as a standard reference and an ethanol (90%) impregnated discs were used as a negative controls. The plates were incubated at 37^{o} C for 24 hours in inverted position to estimate antibacterial activity against selected bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* (Table 13).

Table 13. Zones of inhibitions as shown as aqueous solution of the ethanolic plant extracts at different concentrations against selected microorganisms (after Borah *et al*. 2012).

Extracts/ Positive control	***Fragaria vesca* extract**		**Ciprofloxacin**	
Concentration	**1mg/ disc**	**0.5mg/ disc**	**5µg/ 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 1mg 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 phenolic extracts on metallo-beta-lactamase, MBL VIM-2 producers *Pseudomonas aeruginosa* clinical strain isolates. Initially *Fragaria vesca* leaves were extracted with ethanol creating 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 determination of MICs of extracts against MBL VIM-2 producers *Pseudomonas aeruginosa* standard microplate assays were used.

The tested extracts presented MICs as follows: crude extract (MIC50 = 10.35 mg/ml, MIC90 = 20.7 mg/ml); fraction Fa (MIC50 and MIC90 = 25 mg/ml); fraction Fb (MIC50 = 6.25 mg/ml, MIC90 = 12.5 mg/ml); fraction Fc (MIC50 and MIC90 = 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 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 /disc. No activity of the extract against *Mycobacterium avium* at 50 µg extract /disc has been established.

Antioxidant activity

In vitro Experiments

Fragaria vesca water extract

The total antioxidant capacity of 70 plants, *Fragaria vesca* leaves included, was estimated by Ferric Reducing/Antioxidant Power (FRAP) assay by Katalinic *et al* (2006). The total phenolic content of the leaves infusion 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 .06 to 25 mM/L. There was significant linear correlation between total phenolic content and FRAP. *Fragaria vesca* leaves infusions were prepared according to a standard protocol. To 3 g of plant material was added 200 mL of deionised water. The initial temperature of added water was 98 C. Infusion was left to stay at room temperature without additional heating for 30 min. The extract was filtered and the liquid portion was analyzed 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* leaves infusate (Katalinic *et al*. 2006).

Plant material	Total phenolics (mg CE/L)[a]	FRAPb (Imol/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).

Antioxidant properties of Fragariae herba folium, when measured with the FRAP assay was comparatively strong and had a significant reducing power and free radical scavenging ability *in vitro* comparable with red wine or beverages like tea (*Camellia sinensis*).

Buricova *et al*. (2008; 2011) tested antioxidant activity of some substances present in medicinal plants, the wild strawberry leaves included. For the water extracts preparation, the leaves of *Fragaria vesca* were ground and 1 g of the ground leaves was left in 50 ml of deionised water for extraction during 20 minutes. The temperature of the water was 98°C. For these studies 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. Determination of selected antioxidants was performed by HPLC method (Table 15; Table 16).

Table 15. Antioxidant activity of *Fragaria vesca* water extract determined using four methods and their comparison with the antioxidant capacity of 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 FeSO4/l; ORAC in μmol Trolox/g of dry sample; TPC in mg gallic acid/g of dry sample

Table 16. Concentration of antioxidants in the leaves of the water extracts and their participation (% AC) in 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 (TPC, DPPH, ORAC, FRAP) of the studied water extract of the wild strawberry leaves were determined to be in the range about 50 % of the antioxidant capacity of green tea water extract, and good antioxidant capacities of the studied herbs infusions (determined by DPPH method) were observed in comparison with those of tea infusions, wines, and other beverages. Moreover (+)-

catechin, ellagic acid, and (–)-epicatechin significantly participated in the antioxidant activity of the leaves water extract of the wild strawberry leaves.

Interestingly, in the ethanolic extract of *F. vesca* leaves extracts six phenolic compounds – (+)-catechin, (−)-epicatechin, ellagic acid, epigallocatechin gallate, hyperoside, isoquercitrin and three quercetin derivatives were identified. Fragaria leaf extracts exhibited strong antioxidant activity (range of total TEAC values 191.23-609.36 _mol/g and 178.63-642.20 mol/gof ABTS and FRAP respectively) (Raudonis et al. 2012).

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

Herbal preparation tested	Strength Dosage Route of administration	Experimental model *In vivo*/ *In vitro*	Reference Year of publication	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 differences between the different extracts in inhibiting the enzyme, and respectively: 13.9 ± 1.1; 53.6 ± 4.2 and 56.0 ± 0.3
1. Hydroalcoholic extract of Fragaria vesca leaves. 2. Aqueous extract of Fragaria vesca leaves.	80 and 160 mg/mL	In vitro Experiments. Effect of Fragaria vesca leaves hydroalcoholic extract 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 (80and160 mg/mL) the extract inhibited nitrite production, probably due to a direct nitric oxide scavenging.
Fragaria vesca leaves methanol extract Fragaria vesca leaves acetone extract Five glycoconjugates composed of carbohydrates and phenolic and protein constituents : Fv I – FvV	Concentrations of a sample in the clotting mixture tested in the range from 4000 to 7.81 µg/mL	*In vitro* Experiments Anticoagulant activity in human plasma measured by activated partial thromboplastin time test , prothrombin time test, and thrombin time test	Pawlaczyk et al. 2009; 2013	All Fragaria vesca isolates displayed the anticoagulant activity, in the potency order Fv I > Fv III > Fv II > Fv IV > Fv V. Only two glycoconjugates, i.e. Fv III and Fv I have been shown to have a significant biological activity, but lower than that of unfractionated heparin.

Herbal preparation tested	Strength Dosage Route of administration	Experimental model *In vivo*/ *In vitro*	Reference Year of publication	Main non-clinical conclusions
Anticancer and antithrombin activity *In vitro* Experiments Fragaria vesca methylene chloride extract Fragaria vesca methanol extract	50 µl of plant extracts was used	*In vitro* Experiments Antithrombin bioassay was performed according the method of Medeiros et al. (2000). Anticancer activity of the tested extracts were tested on mouse leukemia cells (L1210).	Goun et al. 2002	Antithrombin activity of Fragaria vesca extracts was in the range of 77% of inhibition
Fragaria vesca leaves aqueous extract	Following concentrations of the extract were used: 0.06, 0.6, 6 and 60 mg/100 ml.	Vasoactive effects. 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 cyclooxygenase inhibitor indomethacin	Mudnic et al. 2009.	Maximal 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 antagonized by the highest dose (60.0 mg/100 ml) of the extract. Received results indicate, that the vasodilatory effect of the wild strawberry leaves aqueous extract is endothelium dependant.
Fragaria vesca leaves aqueous extract In vitro experiments	Concentrations of 0.06, 0.18, 0.6, and 1.8 mg of the xtract /100 ml	Cardiac effects Guinea pig isolated hearts *In vivo*	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%.

Herbal preparation tested	Strength Dosage Route of administration	Experimental model *In vivo*/ *In vitro*	Reference Year of publication	Main non-clinical conclusions
Fragaria vesca leaves ethanolic extract	1mg of the extract/ disc; 0.5mg of the extract/ disc	Antimicrobial activity *In vitro* experiments Estimation of the antibacterial activity against selected bacterial strains: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa	Borah et al. 2012	Significant antibacterial effect has been found for the Fragaria leaves ethanolic extract (0.5 mg and 1mg samples) against *Staphylococcus aureus* and *Escherichia coli* strains. The *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	The tested extracts presented MICs as follows: crude extract (MIC50 = 10.35 mg/ml, MIC90 = 20.7 mg/ml); fraction Fa (MIC50 and MIC90 = 25 mg/ml); fraction Fb (MIC50 = 6.25 mg/ml, MIC90 = 12.5 mg/ml); fraction Fc (MIC50 and MIC90 = 12.5 mg/ml). The most active antibacterial fraction was the Fb fraction consisting essentially of tannins: proanthocyanidins and ellagitannins.
Fragaria vesca leaves Methanol extract	50 µg extract / disc.	*In vitro* Antimycobacterial effects of the methanol extract	McCutcheon *et al.* 1997 (cited after Newton *et al*. 2000).	Methanol extract induced of small zone of clearing of *Mycobacterium tuberculosis* at 50 µg extract /disc. No activity of the extract against *Mycobacterium avium* at 50 µg extract /disc has been established.

Herbal preparation tested	Strength Dosage Route of administration	Experimental model *In vivo*/ *In vitro*	Reference Year of publication	Main non-clinical conclusions

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 1g/kg in rats (oral administration) but showed no significant influence in diuresis. However, the reported pharmacological effects are consistent with the traditional use.

The astringent effect of strawberry may be due to the high tannins content (at least 3.0 percent 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

The are not data available on the acute toxicity of Fragaria leaves

3.3.2. Repeated dose toxicity

Subacute, Chronic Toxicity

There are not data available.

3.3.3. Genotoxicity

Mutagenicity

There are not 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 justifies and confirms the reported astringent and anti-inflammatory activity of the wild strawberry leaves. Results from relevant non-clinical experimental studies are scant, but the astringent, anti-inflammatory, antimicrobial and antihadesive 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.
Adequate 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 authorised products according to Article 10a of Directive 2001/83/EC 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 well documented in several handbooks throughout a period of at least 30 years (15 years in the European Community) under Directive 2004/24/EC and thus, it can be considered as safe when used in recommended dosages under the conditions specified in the monograph.

Several preparations and posologies for *Fragaria vesca, F. moschata, F. viridis* and *Fragaria x ananassa* leaves are included in handbooks, mainly aqueous preparations (infusion and decoction) for oral use, mainly to to increase the amount of urine and to treat mild or acute diarrhea. These indications are not acknowledged by preclinical or clinical studies. Those preparations including relevant information regarding preparation and dosage have 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 therefore 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 the wording accepted for other 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 Community list entry for *Fragaria vesca* , *Fragaria moschata, Fragaria viridis* and *Fragaria x ananassa* cannot be recommended.

Annex

List of references