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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Ribes nigrum* L., 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)	*Ribes nigrum* L., folium
Herbal preparation(s)	a) Comminuted herbal substance b) Dry extract (DER 7:1), extraction solvent water c) Powdered herbal substance
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use. Herbal preparations in solid dosage forms for oral use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term.
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Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Ribes nigrum* L., 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

In accordance with the European Pharmacopoeia (8th ed., 07/2013:2528) blackcurrant leaf consists of the dried leaves of *Ribes nigrum* L. (blackcurrant leaf). The leaf is simple, lamina is up to 10 cm long and 12 cm wide, with 3 or 5 rounded triangular lobes and the median lobe is the largest (European Pharmacopoeia 8th ed., 07/2013:2528).

Description and origin of the plant

Ribes nigrum L. belongs to the family of the *Grossulariaceae*. The genus *Ribes* contains between 140 and 150 species. The leaves are collected during or shortly after flowering (Pharm. Française 1996; Hänsel *et al*., 1994). A dark green upper surface and a pale greyish green lower surface is characteristic for the slightly wrinkled leaf fragments. Furthermore, a widely spaced reticulate venation is particularly distinct on the lower surface. Glands can be seen as scattering yellowish dots. In contrast with the fresh leaves, the dried leaves have no odour or taste (Wichtl 1994; Hänsel *et al*., 1994).

Constituents

The dried *Ribes nigrum* leaf contains not less than 1% of flavonoids, expressed as isoquercitroside. The material complies with the monograph of the European Pharmacopoeia 8th ed. (2013) and British Pharmacopoeia Vol IV (2015). According to the Pharmacopée Française the dried *Ribes nigrum* leaf contains not less than 1.5% of flavonoids, expressed as rutin (ESCOP, 2003).

The most important secondary metabolites present in the herbal substance can be subdivided into several groups of phytochemical compounds.

Polyphenolic substances, more particularly flavonoid glycosides: kaempferol, quercetin, myricetin, isorhamnetin and sakuranetin (Wyk & Wink, 2005).

Some substances can seasonally appear in the glands of leaves of *Ribes nigrum*. Sakuranetin is a methylated flavanone aglycone (4',5-diOH-7-methoxyflavanone). Its biosynthesis may be useful in the protection against parasites like *Botrytis cinerea* Pers. ex Fr. (Atkinson & Blakeman, 1982).

Hydroxycinnamic acid derivatives: chlorogenic acid and chlorogenic acid derivatives (isochlorogenic acid, neochlorogenic acid), caffeic acid, gallic acid, ferulic acid, coumaric acid, gentisinic acid (Trajkovski, 1974a; Trajkovski 1974b).

Prodelphinidins (**proanthocyanidines)** were identified in a methanolic extract of the leaves. They may be responsible for the anti-inflammatory properties of the herbal preparations (Tits *et al*., 1992a, 1992b).

The presence of **glycerolipids** has been reported. The total fatty acid composition was unusual, because the following unsaturated fatty acids were identified: linolenic acid (alpha-18:3), together with cis-7, 10, 13-hexadecatrienoic acid (16:3) and lower amounts of stearidonic acid (18:4) and gamma-linolenic acid (gamma-18:3). This makes the lipid composition type mixed: typical of 16:3 plants but also partially typical for 18:4 plants (Dobson, 2000).

The essential oil of the leaves of *Ribes nigrum* contains mainly monoterpenic substances like alpha-pinene, myrcene, p-cymene, limonene, beta-ocimene, beta-phellandrene, linalool, terpinen-4-ol,

geraniol, citronellylacetate. Furthermore, the sequiterpenes caryophyllene and humulene were identified, as well as methyl salicylate (Andersson *et al*., 1963).

Ascorbic acid, carotenoids (Herbal Medicines, 2013).

Maximum content of Ca, Mg, Fe, Al, Cr and K in the black current leaves is detected in the June (Nour *et al*., 2014).

The potassium-sodium ratios in the leaf of *Ribes nigrum* L. and decoctions of the leaves were 128:1 and 242:1 respectively. These ratios are considered as eventually contributing to the diuretic effect (Szentmihályi *et al*., 1998).

- Herbal preparation(s)

The information about the currently registered/authorised herbal preparations on the European market of Ribis nigri folium was provided by the National Competent Authorities in the overview of the market products (see section 2.1.1.).

In France, Spain and Poland the following herbal preparation of Ribes nigrum L., folium is present as monocomponent medicinal products:

- comminuted herbal substance for tea preparation
- dry extract (DER: 7:1; extraction solvent water) in solid dosage form as hard capsule
- powdered herbal substance in solid dosage form as hard capsule

Table 1: Information obtained from pharmacopoeias and handbooks

Reference, year	**Herbal preparation**
Herbal Medicines (Fourth edition), 2013	As tea of finely cut dried leaves for infusion, 2-4 g per cup (150 mL) three to four times daily; Dry extract (7:1, water), 169 mg per capsule, 1 -3 capsules daily; Powdered herbal substance, 340 mg per capsule, three times daily.
Hänsel *et al*., 1994; Delfosse, 1998; Wichtl, 1994; Wyk & Wink, Gruenwald *et al*., 2000	As tea of finely chopped leaves, 2-4 g taken several times a day.

- 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.

In Hungary Ribis nigri folium is available as a component of combination medicinal products in the old ‘healing products’ category with the indication: prevention of cardiac complaints in elderly, relieve symptoms of temporary nervous cardiac complaints. This information was provided by the National Competent Authority in the overview of the marketed products.

This assessment report and the Community herbal monograph refers exclusively to Ribis nigri folium as a single ingredient. The Community herbal monograph describes the use of the comminuted herbal substance for tea preparations, powdered herbal substance in solid dosage form as hard capsule and dry extract in solid dosage form as hard capsule.

Vitamin(s): not applicable

Mineral(s): not applicable

1.2. Search and assessment methodology

Electronic databases and other sources used to assess information available on traditional use, pharmaceutical, non-clinical, clinical data and current indications on *Ribes nigrum* L., folium using search keywords: *Ribes nigrum* L., Ribis nigri folium, Blackcurrant leaf, cassis. No restrictions to language were set. The search of information was performed from June till September 2016.

Articles and references were retrieved from:

- Scientific databases: PubMed, ScienceDirect, Scopus, Scifinder, Web of Science, EMBASE, EBSCO.
- Medical databases: UpToDate.
- Toxicological databases: TOXLINE.
- The Cochrane Library: 1 reference was found using *Ribes nigrum L*., folium or Blackcurrant leaf as search terms.
- Libraries: hand searches in handbooks, textbooks and Pharmacopoeias on *Ribes nigrum L*. folium and Blackcurrant leaf at the various libraries: EMA, University of Latvia, Rigas Stradinu University, The State Agency of Medicines of Latvia, National library of Latvia.
- Databases of electronic books: Dawsonera.
- Search engines used: Google, Google Scholar.

Other databases: The World Health Organization, National Center for Complementary and Alternative Medicine (NCCAM).

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

Table 2: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Ribes nigrum L., folium Dry extract (extraction solvent water; DER: 7:1)	a) Traditionally used to treat minor articular pain. b) Traditionally used to support renal and digestive elimination systems.	Herbal preparations in solid dosage form: hard capsule Method of administration: oral use Posology: adults only Single dose: 169 mg of extract/hard capsule	France TUR From 1990 until 2011 it was authorised / registered in France as a medicinal product. Currently there are no monocomponent

Active substance	Indication	Pharmaceutical form	Regulatory Status
		Daily dose: 169 mg of extract/hard capsule 1 to 3 times daily Duration of use: a) 4 weeks b) 2-3 weeks	medicinal products containing *Ribes nigrum* L., folium as dry extract authorised/registered in France
Ribes nigrum L., folium Powdered herbal substance	a) Traditionally used in the symptomatic treatment of minor painful joint conditions. b) Traditionally used to promote urinary and digestive elimination functions.	Herbal preparations in solid dosage form: hard capsule Method of administration: oral use Posology: adults only Single dose: 1 hard capsule contains 340 mg of powder Daily dose: 1 hard capsule 3 times daily Max dose: till 5 hard capsules if necessary Duration of use: 4 weeks 2-3 weeks	TUR use in **France** since 1987
Ribes nigrum L., folium Powdered herbal substance	a) Traditional herbal medicinal product for relief of minor articular pain. b) Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.	Herbal preparations in solid dosage form Method of administration: oral use Posology: adults only Single dose: 1 hard capsule contains 340 mg of powder Daily dose: 3 capsules per day, 1020 mg Duration of use: 2 to 4 weeks 2 to 4 weeks	Spain It was registered by former registration scheme in January 1992. TUR since May 2009, according to article 16 of Directive 2001/83 was granted.
Ribes nigrum L., folium Comminuted herbal substance	Traditionally used as an aid in mild rheumatic complaints.	Herbal preparations for infusion as herbal tea Method of administration: oral use Posology: adults only Daily dose: 2 to 4 g	TUR use in **Poland** since 1978

Active substance	Indication	Pharmaceutical form	Regulatory Status
		pour with 200mL of boiling water, infuse under cover for 10-15 min. Drink freshly prepared infusion 3 times a day. Duration of use: 4 weeks	

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

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

Hungary

Combination product

230 g of the preparation contains:

Crataegi extractum (extraction solvent red vine + alcohol):	178.71
Crataegus spp., folium cum flore	
Ribes nigrum L., folium	
Crataegus spp., fructus	
Melissae herba	
Kalium asparticum	0.46 g
Magnesium asparticum	0.23 g

Indication: prevention of cardiac complaints in elderly, relief of symptoms of temporary nervous cardiac complaints (e.g. palpitations, perceived extra heart beat due to mild anxiety).

All together there are six combination products containing *Ribes nigrum* L., folium in the old 'healing products' category.

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

Latvia

The herbal preparations that contain *Ribes nigrum* L., folium are available on the market as food supplements (combination products).

Conclusion about products on the market in the EU/EEA Member States

According to the information provided by the National Competent Authorities in the overview of the marketed products, mono-component medicinal products, containing *Ribes nigrum* L., folium, are available for 30 years in markets of France, Poland and Spain therefore fulfilling the criteria of traditional use in EU.

Combination products containing *Ribes nigrum* L., folium are available in Hungary in the old 'healing products' category.

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

Not applicable

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

Inflammatory conditions

Indications exclusively in folk medicine for the *Ribes nigrum* leaf are: arthritis, rheumatic complaints, diarrhoea, spasmodic cough as well as it has been traditionally used as an infusion to cure joint complaints (traditionally classified as 'rheumatism') (Wichtl, 1994; Leclerc 1983; Rombi, 1991). The only indication mentioned in the ESCOP monograph is 'adjuvant in the treatment of rheumatic conditions' (ESCOP, 2003).

Anti-inflammatory posology according to ESCOP (2003), Van Hellemont (1985) and Delfosse (1998):

- Dried leaves as an infusion: from 1.5 to 4 g per cup (= 150 mL; 3-4 cups daily) to 20-50 g per litre (250 to 500 mL daily), infused during 15 minutes.
- Fluid extract (1:1): 5 mL 2x daily. The extraction solvent is not specified.

Ribis nigr*i* folium is also described in folk medicine as a diuretic, eliminating uric acid. The preparations mentioned are aqueous decoctions (Decaux, 1930; Hänsel *et al.*, 1994).

Some authors mention the use of tea preparations containing *Ribes nigrum*, *Fraxinus excelsior* and *Ulmaria officinalis*.

According to some authors, the essential oil of *Ribes nigrum* stimulate the renal epithelium and enhance diuresis (Garnier *et al*., 1961; Rombi, 1991). Rarely preparations of Ribis nigri folium were locally applied on wounds (Wichtl, 1994), as well as the leaves were applied on the head against migraine (Decaux, 1930).

Table 3: Overview of historical data

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form**
Comminuted herbal substance or equivalent preparations	Adjuvant in the treatment of rheumatic conditions	20-50 g of dried leaf per 1 L, infused for 15 minutes. Daily dosage: 250-500 mL; Fluid extract (1:1) 5 mL Daily dosage: twice daily. Taken before meals.
Comminuted herbal substance or equivalent preparations	Traditional diuretic. Leaves are taken in cases of rheumatism and urinary problems	As a tea, 2-4 g of finely chopped leaves taken several times a day

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form
Comminuted herbal substance or equivalent preparations	Usage based on tradition in folk medicine: as diuretic, uses for gout, rheumatic complaints, diarrhoea, spasmodic cough.	Single dose: 2-4 g of finely cut dried leaf, pour boiling water over and steep for 5-10 minutes and then strain. 1 cup several times daily.
Comminuted herbal substance or equivalent preparations	Usage based on tradition in folk medicine: arthritis, gout and rheumatism, diarrhoea, colic, jaundice and liver ailments, painful micturition, urinary stones, convulsive coughs and whooping cough.	Single dose: 2-4 g of black current leaves in boiling water (150 mL) for the tea, strain after 15 min. preparation. Daily dosage: 1 cup to be drunk several times a day.
Comminuted herbal substance or equivalent preparations	Usage medicine: arthritis, gout and rheumatism, diarrhoea, colic, jaundice and liver ailments, painful micturition, urinary stones, convulsive coughs and whooping cough, joint pain, diuretic	Single dose: 2-4 g /150 mL in boiling water over and steep for 10 min and then strain. Daily dosage: 3-4 cups to be taken several times a day.
Comminuted herbal substance or equivalent preparations Powdered herbal substance Dry extract (7:1, water)	For minor articular pain and minor urinary complaints (as a diuretic)	Dry extract (7:1, water), Single dose: 169 mg per capsule. Daily dosage: 3 capsules daily. Powdered herbal substance Single dose: 340 mg per capsule. Daily dosage: up to 5 capsules daily. Infusion as herbal tea of dried leafs: 2-4 g per cup (150 mL). Daily dosage: 3-4 times daily. Fluid leaf extract (1:1) Single dose: 5 mL. Daily dosage: 2 daily before meals.

2.3. Overall conclusions on medicinal use

Herbal tea (2-4 g up to 3 times daily) has been used since 1978 in Poland for minor rheumatic complaints and can be accepted according to the 30 years of use stipulated in Directive 2004/24/EC.

Powdered herbal substance in capsules has been marketed in France (since 1987 and in Spain (since 1992) in different posologies (France: 340 mg up to 5 capsules per day; Spain: 340 mg 3 times daily). At time of monograph systematic review (in 2016) the period of use fulfils the 30 years of traditional use as stipulated in Directive 2004/24/EC, therefore, this preparation can now be included in the monograph.

Dry extract (7:1; extraction solvent water) is marketed in France as capsules (169 mg per capsule; posology: up to 3 capsules daily) meets the 15-year tradition in Europe and does not comply with the 30 years of traditional use as stipulated in Directive 2004/24/EC. Nevertheless, during its meeting of 12 January 2010 the MLWP decided to accept this preparation based upon the following justification:

- The extract is made with water, a procedure comparable to herbal tea preparations.

- The drug-extract ratio is 7 to 1 and 169 mg is taken as a single dose up to 3 times a day. One capsule corresponds to 1183 mg of herbal substance. For herbal tea, 2 to 4 g herbal substance is infused; virtually, more material can be extracted by preparing the herbal tea. The number of daily doses is similar for the extract and the infusion.

Table 4: Overview of evidence on period of medicinal use

Herbal preparation	**Indication**	**Posology, Strength**	**Period of medicinal use**
Comminuted herbal substance	Traditionally used as an aid in mild rheumatic complaints	Herbal preparations for infusion as herbal tea Posology: adults only Dose: 2 to 4 g in 200mL of boiling water, infuse for 10-15 min. Drink freshly prepared infusion 3 times a day. Method of administration: oral use Duration of use: 4 weeks	TUR use in **Poland** since 1978
Powdered herbal substance	a) Traditionally used in the symptomatic treatment of minor painful joint conditions. b) Traditionally used to promote urinary and digestive elimination functions.	*Adults* Herbal preparations in solid dosage form: hard capsule Posology: Single dose: 1 hard capsule contains 340 mg of powder Daily dose: 1 hard capsule 3 times daily	TUR use in **France** since 1987 (and TUR use in **Spain** since May 2009)

Herbal preparation	Indication	Posology, Strength	Period of medicinal use
		Max dose: till 5 hard capsules if necessary (in France) Method of administration: oral use Duration of use: 4 weeks 2-3 weeks	
Dry extract (extraction solvent water; DER: 7:1)	a) Traditionally used to treat minor articular pain. b) Traditionally used to support renal and digestive elimination systems	Herbal preparations in solid dosage form: hard capsule Posology: adults only Single dose: 169 mg1) of extract/hard capsule Daily dose: 169 mg1) of extract/hard capsule 1 to 3 times daily Method of administration: oral use Duration of use: a) 4 weeks b) 2-3 weeks	Currently there are no monocomponent medicinal products containing *Ribes nigrum* L., folium as dry extract authorised/registered in France as TUR It was registered in France as a medicinal product from 1990 until 2011

[1)] In the monograph this value is changed into 170 mg.

The use of leaves of *Ribes nigrum* in children and adolescents under 18 years of age is not recommended due to lack of data.

Because the HMPC could not find a suitable indication for the traditional use "to support digestive elimination", this use was not included in the monograph.

Therefore, the following indications for *Ribes nigrum* L., folium are proposed for the Community Monograph:

Traditional herbal medicinal product based upon long-standing use

Indication a): Traditional herbal medicinal product for relief of minor articular pain.

In adults

- Comminuted herbal substance for infusion as herbal tea preparation

Single dose: 2 to 4 g per cup, 3 times daily.

Daily dose: 6 – 12 g.

- Dry extract (DER 7:1, water)

Single dose: 1170 mg, 1 - 3 times daily.

Daily dose: 510 g.

- Powder in hard capsule

Single dose: 340 mg, 3 -5 times daily.

Daily dose: 1020 – 1700 mg (3 – 5 hard capsules per day)

Duration of use: 4 week

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

In adults:

Herbal preparations in solid dosage form: hard capsule

- Dry extract (DER 7:1, water)

Single dose: 170 mg, 1-3 times daily.

Daily dose: 510 g.

- Powder in hard capsule,

Single dose: 340 mg, 3-5 times daily

Daily dose: 1020 – 1700 mg (3-5 hard capsules per day)

Duration of use: 2 week

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

Analgesic effects

In vivo studies

Ethanolic extracts

Mongold *et al.,* (1993) reported that a *Ribes nigrum* L., folium extract (dried 500g leaves immersed in 15% ethanol/water in 5 L) for 10 days, then filtered and lyophilised) induced a analgesic effect in the acetic-induced writhing test in mice as well as in the Hot-plate responce test in mice.

Anti-inflammatory activity

In vivo studies

Ethanolic extracts

Anti-inflammatory activities were observed by Mongold *et al.,* (1993) in several *in vivo* models:

a) in the carrageenan-induced acute inflammation model *Ribes nigrum* L., folium extract (extraction solvent: 15% ethanol), demonstrated dose-dependent (75 and 150 mg/kg, IP) inhibition of acute inflammatory oedema, the effect of 150mg/kg dose was comparable to indomethacin (5 mg/kg, IP)

b) cotton pellet granuloma: *Ribes nigrum* L., folium extract (150 mg/kg, IP) inhibited granuloma formation – the relative wet granuloma weights for the control (465±19 mg), *Ribes nigrum* L., folium extract (351±15 mg) and indomethacin (378±8 mg);

c) Freund adjuvant induced arthritis: the *Ribes nigrum* L., folium extract (150 mg/kg – 18.71%; 300 mg/kg – 34.65%) expressed dose-dependent reduction of hind-paw oedema.

Other extract (maceration of 60 g black currant leaves with 1000 mL of 14% ethanol; 1 mL/kg and 10 mL/kg, *per os*) of *Ribes nigrum* L., folium demonstrated the anti-inflammatory activity by the inhibition of carrageenan-induced inflammation compared to reference compounds indomethacin and niflumic acid in acute and chronic studies in *Sprague-Dawley* rats (Declume *et al.,* 1989).

Ribes nigrum L., folium extract (containing 60% of proanthocyanidins of the total polyphenol content) increased the CD39-positive endothelial cell fraction in a concentration-dependent manner (for the 2.5 µg/mL it increases up to 10%; for 15 µg/mL up to 33%). It also enhanced endothelial nitric oxide synthase (eNOS) activation. T495 phosphorylation was decreased by 31±6% for the dose of 2.5 µg/mL and 48±6% for 15 µg/mL, whereas S1177 phosphorylation increased by 13±3% for the dose of 2.5 µg/mL and 18±7% for 15 µg/mL compared to untreated cells (Luzak *et al.,* 2014).

Anti-inflammatory activity of fresh *Ribes nigrum* L., folium extract (extraction solution: acetone/water/acetic acid (70:28:2)) was tested on the total myeloperoxidase (MPO, dose 50 ng/mL) released by activated neutrophils measured by an ELISA assay. The higher concentration (1 mg/mL) of *Ribes nigrum* L., folium gave about 15-20% of inhibition. MPO inhibition was also observed in a Specific Immunological Extraction Followed by Enzymatic Detection assay, *Ribes nigrum* L., folium extract showed a significant dose-dependent inhibition: the lowest a concentration tested, 0.5 µg/mL for leaf extract, showed 60% inhibition (Tabart *et al.*, 2012).

Isolated constituents

In vitro studies:

Shiba *et al.,* (2008) observed that flavonoids (quercetin, and its metabolites) inhibited the formation of dityrosine catalysed by the myeloperoxidase (MPO) enzyme in a dose-dependent manner (1,.25 – 100 µM) *in vitro* (HL-60 cells).

The experimental model consisted of an LT2 cell line originating from human umbilical vein endothelium cells. A proanthocyanidin-enriched fraction was obtained from leaves from *Ribes nigrum* with acetone extraction (70% v/v in water). Purification was done on reversed phase chromatography.

A significant inhibition of TNF-α (Tumor Necrosis Factor) stimulated ICAM-1 (Intercellular AdhesionMolecule 1) expression but not IL-8 and VEGF155 mRNA expression was observed with proanthocyanidins in concentrations from 10 µg/mL to 60 mg/kg (Garbacki *et al.,* 2005). An *in vitro* decrease of PGE2 production in human Chondrocytes was also observed with prodelphinidines, obtained from the *Ribes nigrum L.*, folium, A concentration of 10^{-4}M of gallocatechin and its dimer inhibited the formation of prostaglandins comparable with indomethacin 10^{-5}M: 53%, 57% and 67% respectively. Further, the selectivity on COX-2 inhibition was confirmed (Garbacki *et al.*, 2002).

Ex vivo studies:

An anti-inflammatory activity is reported for rutin and isoquercitrin, obtained from the *Ribes nigrum* L., folium (Chanh *et al*., 1986).

In vivo studies: Anti-inflammatory effects of proanthocyanidins (PACs) were observed in rat's models of carrageenan induced paw oedema and carrageenin-induced pleurisy. Pretreatment with PACs (10, 30, 60 and 100 mg/kg, i.p.) reduced in a dose time dependent manner paw oedema induced by carrageenin and also inhibited carrageenin-induced pleurisy in rats: particularly reducing lung injury, pleural exudate formation, polymorphonuclear cell infiltration, pleural exudate levels of TNF-α, IL-1β and CINC-1, pleural exudate levels of nitrite/nitrate (NOx). The mechanism of action of the PACs differs from that of indomethacin. Indomethacin treated rats showed that a low the volume of pleural

exudate , and a reduced content in leukocytes and in TNF-α, IL-1β, IL-6 and IL-10 but not in NOx (Garbacki *et al.*, 2004).

Garbacki *et al.*, (2005) observed anti-inflammatory activities of proanthocyanidin-enriched fraction (PACS) of *Ribes nigrum* L., folium (the extraction solvent was acetone (7O% v/v in water purification by reversed phase chromatography) in Wistar rats with a dose of 10, 30 and 60 mg/kg/per animal). The following effects were observed:

a) a dose-dependent inhibition of the carrageen-induced pleurisy by reducing pleural exudate formation and PMNs infiltration;

b) leukocyte cell adhesion molecules mobilization was not down-regulated on granulocytes;

c) a decrease in the production of endothelial cell adhesion molecules on the lung sections.

Rodelphinidins (5, 10, 40 and 60 mg/kg) isolated from of dried *Ribes nigrum* L., folium extract demonstrated anti-inflammatory activities in a rat carrageenan paw oedema model by reducing paw oedema 18% for 5 mg/kg; 40% -10 mg/kg and 55% - 40 mg/kg, whereas reference compounds indomethacin 4 mg/kg reduced it by 44% and aspirin 200 mg/kg by 47% (Tits *et al.*, 1991).

Diuretic activity

In vivo studies:

The model used was a salidiuretic action. The intervention consisted of oral administration of a fluidextract (extraction solvent was ethanol; 1:1) of blackcurrant leaf in rats. The diuretic action of an equivalent of 1500 mg dried leaf/kg was comparable to the effect of furosemide at 50 mg/kg (Rácz-Kotilla & Rácz 1977).

Table 5: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Ribes nigrum L., folium (dried) extract Extraction solvent: 70% (v/v) aqueous acetone	0.25-15 µg/mL	In vitro Human umbilical vein endothelial cells	Luzak *et al.*, 2014	The anti-inflammatory activity demonstrated by increasing the CD39-positive endothelial cell fraction and enhanced endothelial nitric oxide synthase activation
Ribes nigrum L., folium extract, extraction solvent: 1 g of fresh leaves was	Extracts concentrations:5 0, 25, 10, 7.5, 5, 2.5, 1, 0.5 µg mL^{-1}	*In vitro:* neutrophils The oxidant response of neutrophils and on myeloperoxidase	Tabart *et al*., 2012	The anti-inflammatory activity demonstrated by inhibition of myeloperoxid

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
ground with 1 g of quartz and 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)		(MPO) activity; effects on the release of MPO by stimulated neutrophils; effects on the specific activity of MPO measured by The Specific Immunological Extraction Followed by Enzymatic Detection (SIEFED)		ase activity and ROS production on activated neutrophils
Ribes nigrum L., folium extract, extraction solvent: 15% ethanol, lyophilised	a) Writhing induced by acetic acid; b) Hot-plate response in mice: Ribes extract 200 mg/kg, IP and morphine 4 mg/kg, IP	*In vivo*: <u>Swiss mice</u> a) Writhing induced by IP injected 0.75 % acetic acid aolution (10 mL/kg), extract given IP 30 min before acetic acid injection. ED50 estimated: the dose of the drug that reduced by 50% the number of mice exhibiting writhing compared to the control animals; b) Hot-plate response in mice: Ribes extract has shown a peripheral analgesic effect	Mongold *et al.*, 1993	The analgesic effects demonstrated in Writhing induced by acetic acid test, Hot-plate response test in mice
Ribes nigrum L., folium extract, extraction solvent: 14% ethanol, lyophilised	Extract 1 mL/kg and 10 mL/kg, per os	*In vivo*: Plethysmometric measurements in Sprague-Dawley rats – extract was given per os 30 minutes before carrageenan injection. *Ribes nigrum* L., folium extract (1 and 10 mL/kg) dose-dependently (30% and - 54%, respectively) reduced rat paw oedema	Declume *et al.*, 1989	The anti-inflammatory activity demonstrated by the inhibition of carrageenan-induced inflammation in Sprague-Dawley rats

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
		after 4h, compared to indomethacin (for 2.5 mg/kg was 63% and 5 mg/kg - 66%) and niflumic acid (25 mg/kg was 19% reduction and and 50 mg/kg was 70%) in acute; for the chronic studies (21 and 28 days oral treatment) *Ribes nigrum* L., folium extract at the dose of 0.33, 1 and 10 mL/kg reduction of oedema was 30%, 42.5% and 46% , respectively, for indomethacin (1,66 mg/kg) it was 49% and niflumic acid (12.5 mg/kg) reduction was 53%.		
Ribes nigrum L., folium extract, extraction solvent: 15% ethanol, lyophilised	a) The carrageenan-induced paw oedema: Ribes extract 100 and 200 mg/kg IP or indomethacine (5 and 10 mg/kg). b) Cotton pellet granuloma: Ribes extract 100 mg/kg IP or indomethacine (3 mg/kg), once daily during 7 days, IP. c) Freund adjuvant induced arthritis: Ribes	In vivo: <u>Wistar rats;</u> a) carrageenan-induced acute inflammation: Ribes extract and indomethacine was injected 1h prior to the subplantar injection of 1 % of carrageenan suspension into the left paws of the rat and the volume of the paw up to the ankle joint was measured by plethysmography b) cotton pellet granuloma:granulomatous lessions were made by inplanting two sterilized cotton pellet (30±1 mg) subcutaneously into dorsal region of the rat c) Freund adjuvant	Mongold *et al.*, 1993	The anti-inflammatory activity demonstrated by the inhibition of carrageenan-induced acute inflammation, (paw oedema after 3 hours was reduced 70 % by the extract 100 mg/kg and 77 % by indomethacin); cotton pellet granuloma (weight of granuloma on the day 8 was reduced 18.6 % by the extract

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
	extract 100 and 200 mg/kg IP or indomethacine (3 mg/kg), once daily during 14 days, IP.	induced arthritis: was induced by single injection of Mycobacterium Butyricum (0.05 mL) in the top of the third part of the tail		150 mg/kg and 24% by indomethacin 3 mg/kg); Freund adjuvant induced arthritis (paw volume was reduced by 18.7% for the extract 100 mg/kg, 34.6% by the extract 200 mg/kg and 37.7% by indomethacin 3 mg/kg
Ribes nigrum L., folium liquid extract (Extraction solvent: ethanol; DER 1:1)	Diuretic quotient = 1.56 when 50 mL fluid extract are given in dilution of 3%/kg body weight.	*In vivo* Rats The diuretic effect of an equivalent of 1500 mg/kg dried *Ribes nigrum* L., folium was comparable to the effect of furosemide at the dose of 50 mg/kg.	Rácz-Kotilla & Rácz, 1977	Diuretic activity was comparable to the effect of furosemide at 50 mg/kg
Isolated constituents				
Quercetin and its metabolite quercetin-3-glucuronide	Concentrations 1.25–100 µM	*In vitro*: HL60 cells; *In vivo*: human atherosclerotic aorta	Shiba *et al.,* 2008	The anti-inflammatory activity of quercetin dehydrate and sulfatase H-1 in both models
Isolated prodelphinidines, extracted from the *Ribes nigrum* L., folium, extraction solvent: acetone (70% v/v in water), purified using reversed phase and	Concentrations (1 to 100 µg/mL) of different prodelphinidines	*In vitro:* purified fractions of prodelphinidines were evaluated on the cultivated human chondrocytes from cartilage: a positive effect on the production of proteoglycans with concentrations from 1 to 100 µg/mL; a positive	Garbacki *et al.,* 2002	The anti-inflammatory activity of prodelphinidines was demonstrated in human chondrocytes showing the selectivity on COX2 inhibition

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Sephadex LH20 column chromatography		effect on type II collagen production with concentration from 1 to 100 µg/mL; an inhibitory effect on the prostaglandin E2 (PGE2) production mainly with concentrations from 10 to 100 µg/mL.		
Total flavonoids, rutin and isoquercitrin, extracted from the *Ribes nigrum* L., folium, extraction solvent:ethyl acetate	No further data about the exact composition are available	*Ex vivo:* isolated rabbit hearts; arachidonic acid (100 µg) was used as a substrate. Total flavonoids extracted from the leaves of *Ribes nigrum*, inhibited both biosynthesis and release of PG-like substances. They were more active than rutin and isoquercitin. The IC_{30} were respectively 1.03 + 0.24 mg/mL, 3.76 + 0.24 mg/mL and 2.31 + 0.40 mg/mL	Chanh *et al*., 1986	The anti-inflammatory activity demonstrated for the total flavonoids , rutin and isoquercitrin, obtained from the *Ribes nigrum* L., folium
Prodelphinidins of dried *Ribes nigrum* L., folium, extract, extraction solvent: aqueous acetone, obtained by medium pressure liquid chromatography (MPLC)	Prodelphinidins at the dose of 5, 10, 40 and 60 mg/kg; indomethacin 4 mg/kg; aspirin 200 mg/kg; IP injected	*In vivo*: In carrageenan paw oedema model in rats: reduction paw oedema by prodelphinidins of dried *Ribes nigrum* L., folium was 18% for 5 mg/kg; 40% -10 mg/kg and 55% - 40 mg/kg; indomethacin 4 mg/kg reduced by 44% and aspirin 200 mg/kg by 47%	Tits *et al.*, 1991	The anti-inflammatory activity demonstrated in carrageenan paw oedema model in rats
Proanthocyanidin -enriched fraction (PACS) of *Ribes nigrum* L., folium, extraction	Model 1 proanthocyanidins (PACs) at the doses of 10, 30, 60, 100 mg/kg	*In vivo*: Wistar rats Model 1 Carrageenin induced paw oedema: Pretreatment of	Garbacki *et al.,* (2004)	The anti-inflammatory effect of proanthocyanidins was demonstrated

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
solvent: acetone (7O% v/v in water purification by reversed phase chromatography)	or saline; IP injection. Model 2 PACs at the doses of 10, 30, 60 or 100 mg/kg or indomethacin 10 mg/kg, IP injection. Carrageenan at the dose of 0.1 mL and 10 mg/mL	PACs was done before and 1 h, 2 h and 4 h after the injection of carrageenan. The carrageenin (0.1 mL, 10 mg/mL into the plantar region of the right hind paw) was injected 30 minutes after the PACs. Model 2 Carrageenin-induced pleurisy: pretreated with saline, PACs or indomethacin 30 min before the intrapleural injection of the carrageenin in the, pleural cavity opened after 4 hours		in rat's models of carrageenin-induced paw oedema and carrageenin-induced pleurisy. The main mechanism of this effect of PACs lies in an interference with the migration of the leukocytes and inhibition of *in vivo* nitric oxide release.
Proanthocyanidin-enriched fraction (PACS) of *Ribes nigrum* L., folium, extraction solvent: acetone (7O% v/v in water purification by reversed phase chromatography)	Pretreatment with proanthocyanidins (PACs) at the doses of 10, 30 or 60 mg/kg or saline, injected IP	*In vivo*: Male Wistar rats Carrageenin-induced pleurisy: injection of carrageenin in the right pleural cavity 30 minutes after the test substances, Pleural cavity opened after 4 hours. Measurements: accumulation of exudate volume and PMNs. Flow cytometry: analysis of leukocyte cell adhesion molecules (LFA-1, Mac-1 and VLA-4) mobilization in circulating granulocytes. Immunohistochemistry on lung sections: detection of endothelial cell adhesion molecules (ICAM-I and VCAM-I).	Garbacki *et al.,* (2005)	Anti-inflammatory activity demonstrated in rats by PACS at the doses of 10, 30 and 60 mg/kg/rat
Proanthocyanidin-enriched fraction	0.4, 4, 40, 400, mg/g	*In vitro*: endothelial LT2 cells	Garbacki *et al.,* (2005)	Anti-inflammatory activity

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
(PACS) of *Ribes nigrum* L., folium, extraction solvent: acetone (7O% v/v in water purification by reversed phase chromatography)		stimulated with TNF-α and upon PACs treatment were evaluated for ICAM-1, IL-8 and VEGF mRNA expression		demonstrated in endothelial LT2 cells

3.1.2. Secondary pharmacodynamics

Antioxidative effects

In vitro studies*:*

The polyphenolic fraction of water extract (extraction solvent: water containing 200 ppm of SO2, the ratio of solvent to leaves 3:1) of *Ribes nigrum* L., folium showed antioxidant activity, protecting the pig erythrocyte membrane against free radicals induced by UV radiation (Bonarska-Kujawa *et al.*, 2014). In the study the effect of the extracts on osmotic resistance, shape of erythrocytes was determined with spectrophotometric methods. The result suggest that the extracts of *Ribes nigrum* L., folium protected erythrocytes against the UVC radiation, by the strengthening the membrane and inducing echinocytes (Bonarska-Kujawa *et al.*, 2014).

The antioxidant activity of the polyphenolic (mainly flavonols) extracts of *Ribes nigrum* L., folium in relation to the membrane of erythrocytes and lipids extracted from red blood cell membranes (RBCL) exposed to chemical oxidizing agents (AAPH) was studied fluorometrically, while effects of the extracts on the properties of membranes were examined using calorimetric, IR spectroscopy and fluorimetric methods. According to the authors, the results indicate that the compounds contained in the extracts protect erythrocyte and lipid membranes against oxidation (Cyboran *et al.*, 2014).

A spectrophotometric method was used to investigate the influence of 0.1-0.5 mg/mL of a water extract of *Ribes nigrum* L., folium on osmotic resistance of erythrocytes. On basis of the result the authors conclude that the extract of *Ribes nigrum* L., folium makes the erythrocytes less susceptible to changes in the medium tonicity and may prevent the membrane from stiffening in some pathological states (Cyboran *et al.*, 2012).

Fourteen compounds, including four 7,70-epoxylignans, three tetrahydrofuran-type sesquilignans, and a spirocyclic dilignan, isolated from the leaves of *Ribes nigrum* extract (dried leaves of *Ribes nigrum* (3.0 kg) extracted with ethanol/water (7 L x 3,70:30, v/v)) were evaluated for their antioxidant activities using superoxide anion scavenging assay and DPPH free radical scavenging assay. Of all the compounds tested, ribesin D and ribesin G showed the most potent superoxide anion scavenging activity with EC50 values of 1.24 and 1.12 μM, respectively (Sasaki *et al.*, 2013).

The protective effect of two synthetic antioxidants (quercetin and caffeic acid) and 70% hydroalcoholic extract of blackcurrant leaves was tested in preventing and/or reducing the membrane lipid oxidation due to free radical attack performed by using fluorescence spectroscopy techniques. According to the authors, there results show that natural antioxidants have a much higher antioxidant activity against

free radicals than synthetic compounds but they degrade after two hours of oxidation (Golea *et al.*, 2012).

The antioxidant capacity of the blackcurrant leaf extract (1g of fresh leaves ground with 1 g of quartz in 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)) was tested using several assays. Significant antioxidant activity was seen in the TEAC assay (scavenging of the radical 2,2-azino-bis(3-ethylbenzothiazoline)-6 sulphonic acid, ABTS) - 53.7 ± 6.2 mg TE/g FW. The Cellular Antioxidant Activity of blackcurrant leaves extracts were measured using the CAA assay on EAHy926 cells - CAA value was 12.89 ± 0.77 µmole QE/g of LE. The influence of *Ribes nigrum* L., folium extract on the (reactive oxygen species) ROS production of PMA-activated neutrophils was determined through a lucigenin dependent chemiluminescence assay indicating a significant dose-dependent inhibition, inducing 50% of inhibition with the concentration of 5 ± 0.5 µg of leaf extract/mL (Tabart *et al.,* 2012).

The extract (extraction solvent: water containing 200 ppm of SO2, the ratio of solvent to leaves was 3:1) of *Ribes nigrum* L., folium (0.005-0.05 mg/mL) exhibited an antioxidant activity. It protected the membrane lipids of pig erythrocyte against oxidation, significantly reducing the level of free radicals in erythrocyte suspension detected by the Fluorimetric and Spectrophotometric method. The antioxidant activity of the extract of *Ribes nigrum* L., folium is connected mainly with activity of quercetin lucosides, the extract contains about 77 % of these compounds (Cyboran *et al*., 2011).

Anthelmintic activity

In vivo studies*:*

The development of free-living larvae (*Oesophagostomum dentatum)* was significantly inhibited by *Ribes nigrum* L., folium extract (acetone/water, 3:7) at different concentrations (125, 250, 500 and 1000 µg/mL) (Williams *et al.,* 2014).

Antibacterial activity

In vitro studies*:*

The essential oil (obtained by hydrodistillation) from the *Ribes nigrum* L., folium containing Δ3-carene (18.7%), β-caryophyllene (17.7%), sabinene (11.6%), *cis*-β-ocimene (10.6%) and α-terpinolene (10.6%) showed the antimicrobial activity against 14 micro-organisms (including *Escherichia coli, Streptococcus faecalis*, *Staphylococcus aureus, Candida albicans* and *Trichophyton mentagrophytes* isolates) detected by the broth microdilution method (Stevic *et al*., 2010).

Antiviral effect

In vitro and in vivo studies:

Antiviral activity of a water extract (DER not specified) of *Ribes nigrum* L., folium (0–1 mg/mL) against influenza A virus *in vitro* was observed when the virus was pre-incubated prior to infection or when added directly after infection, however with no antiviral effect when infected cells were treated 2, 4, or 8 h after infection, indicating that the extract blocks a very early step in the virus infection cycle. *In vivo* (the C57BL/6 mouse infection model) study showed that intranasal application of the extract (500 µg) inhibits progeny virus titers in the lung up to 85% after 24 h (Haasbach *et al.*, 2014).

Ehrhardt *et al.* (2013) tested a water extract (DER not specified) of *Ribes nigrum* L., folium against influenza A viruses infections *in vitro* and *in vivo*. The extract inhibited Influenza A virus replication in aconcentration dependent (50, 100 or 200 µg/mL) manner in human alveolar type II epithelial cell line A549 model. The extract did not exert any significant negative effects on cell proliferation or survival, and did not alter transcription or translation processes (Ehrhardt *et al.*, 2013). *Ribes nigrum* L., folium extract (50 and 100 µg/mL) was tested on the A549 cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyltetrazoliumbromide] - Cell Proliferation Assay that is based on an enzymatic reaction of the mitochondrial succinic dehydrogenase. The extract did not affect the cell morphology and viability and did not interfere with cellular proliferation and metabolism (Ehrhardt *et al.*, 2013). In *in vivo* study, BALB/c mice were infected with a sublethal dose of influenza virus A/FPV/Bratislava/79 (H7N7). Further animals were exposed to the same extract as *in vitro* studies: 2 bar of aerosolized extract of *Ribes nigrum* L., folium (prepared from the 10 and 15 mg/mL of stock solutions) at the dose of 1.5 mL/per mouse for 10 min twice a day for three (lung titer) or five (body weight) consecutive days twice a day by using the COAALA Mouse Aerosol Application System. The results showed a reduction of virus titers in the lung of infected animals already at the day three of infection (Ehrhardt *et al.*, 2013).

Antihypertensive effect

In vivo studies:

The model used was the antihypertensive effect on cats. Doses liquid extract (extraction solvent was ethanol; 1:1) equivalent to of 400 mg dried blackcurrant leaf/kg were compared to tolazoline 0.75 mg/kg and 1.0 mg/kg. The antihypertensive effects of both were comparable, but the effect of the leaf extract lasted for 20 minutes as compared to 5 minutes for tolazoline (Rácz-Kotilla & Rácz 1977).

In another study normotensive rats were used. An infusion of blackcurrant leaf (20 g/L) was administered intravenously at a dose equivalent to 360 mg dried leaf per kg. There was a 45% fall in blood pressure, which after 30 minutes was still 30% (Laserre *et al.*, 1983).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Anti-inflammatory, analgesic, diuretic and antioxidative effects of *Ribes nigrum* L., folium extracts and isolated constituents (flavonoids, proanthocyanidins including prodelphinidines) have been demonstrated in the experimental models *in vitro, ex vivo* and *in vivo*.

The preparations tested consisted mainly of alcoholic liquid extracts, which were mostly lyophilised. They were administered per orally as well as intraperitoneally. Several inflammatory parameters were significantly reversed, especially the formation of oedema and the cellular components as illustrated by reduced exudate, infiltration of polymorphonuclear leukocytes, release of interleukins and cytokines and the formation NO-components.

In vitro experiments confirmed the anti-inflammatory actions seen *in vivo*. Flavonoids extracted from *Ribes nigrum* inhibited the biosynthesis of prostaglandins in whole organs (rabbit heart) as did concentrated proanthocyanidines in COX-1 preparations (from ram seminal vesicles) and COX-2 preparations (sheep placenta). The effect was comparable to indomethacin.

Additional properties emerged from *in vitro* experiments with human chondrocytes. Purified proanthocyanidins stimulated the biosythesis of proteoglycans and collagen. This can be considered as an activity complementary to the treatment of inflammation. Furthermore, proanthocyanidins inhibited the formation of tumor necrosis factor-α and the intracellular adhesion molecule in human endothelial cells. Finally, antilipoperoxydase effect can be added to this large scope of activities.

Apart from the anti-inflammatory activity there was also an analgesic activity demonstrated in the acetic acid induced writhing test and the hot-plate response with mice. Alcoholic extracts were intraperitoneally administered.

The medicinal use of *Ribes nigrum* L., folium described in several monographs and well-known handbooks as well as indications of the products available on the EU market are supported by the effects observed in the non-clinical studies.

Based on the available pharmacological data, the following uses are plausible:

- for the relief of minor articular pain;
- to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

The effects not related to the indications proposed for the *Ribes nigrum* L. folium in the monograph but demonstrated in the non-clinical studies are antioxidative effects, antimicrobial, antiviral activity and antihypertensive effect.

None of the reported non-clinical pharmacological studies described indicate a cause for safety concern for the *Ribes nigrum* L., folium.

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

No data with regard to absorption, distribution, metabolism, elimination and pharmacokinetic interactions with other medicinal products are 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

Acute toxicity

Ribes nigrum L., folium liquid extract (1:1) was administered intraperitoneallyto mice. The intraperitoneal LD_0 and LD_{50} were 22 and 49 g/kg respectively. The LD_{100} was estimated at 90 g/kg (ESCOP, 2003).

In another study with mice a lyophilisate obtained by maceration of 100 g leaf per litre 15% ethanol was administered. Intraperitoneal LD_{50} was 1.09 g/kg. Oral doses up to 3 g/kg did not show overt toxicity (ESCOP, 2003; Mongold 1993).

3.3.2. Repeat dose toxicity

Subacute toxicity

Ribes nigrum L., folium was administered to rats as a lyophilised 15% ethanolic extract (1 g of extract was equivalent to 1.8 g of leaf). The extract was administered orally in daily doses of 2 g/kg/day (21 days) and 1.34 g/kg/day (28 days) respectively. No signs of toxicity or gastric ulceration was observed (ESCOP, 2003).

Ribes nigrum L., folium was administered to rats as a lyophilisate obtained by maceration of 100 g leaf per liter 15% ethanol. The extract was administered orally during 10 days without specification of the dose. No change in feeding pattern, fluid consumption or body weight was seen. Blood analysis and

histopathological evaluation of 14 organs did not reveal any abnormalities (ESCOP, 2003, Mongold 1993).

Chronic toxicity

Feeding mice with a daily dose of 3 g/kg of dried leaves during 6 months did not reveal any toxicity (Hänsel *et al.*, 1994).

3.3.3. Genotoxicity

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

The hemolytic activity of the water extract (extraction solvent water containing 200 ppm of SO2) of *Ribes nigrum* L., folium (at the concentrations from the 0,01 up to 0.1 mg/mL) was conducted on fresh, heparinized blood and hemoglobin concentration in the supernatant (expressed as percentage of hemoglobin concentration of totally hemolyzed cells) was assumed as the measure of the extent of hemolysis. At between 0.01 up and 0.1 mg/mL, the extract did not induce hemolysis but protected erythrocytes against the UVC radiation (Bonarska-Kujawa *et al.*, 2014).

Cyboran *et al.*, (2012) also observed that the polyphenols contained a water extract of *Ribes nigrum* L., folium, do not induce hemolysis in concentrations of 0,1-0,5 mg/mL. The Cellular Antioxidant Activity of *Ribes nigrum* L., folium (1 g of fresh leaves ground with 1 g of quartz in 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)) was measured using the CAA assay on EAHy926 cells. Leaf extracts had the highest CAA value (12.89 ± 0.77 µmole QE/g of LE.

Tabart *et al.,* (2012) performed a viability assay using MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltatrazolium bromide; Cell Growth Determination Kit MTT) and found that the *Ribes nigrum* L., folium (1 g of fresh leaves ground with 1 g of quartz in 10 mL of extraction solution: acetone/water/acetic acid (70:28:2)) extracts (0.1 to 1 mg extract/mL) is not cytotoxic on endothelial cells.

In the range of 0.00001–1 mg/mL the water-soluble extract of *Ribes nigrum* L., folium showed no cytotoxic effect on three cell lines (MDCK, A549 and HeLa cells). Cytoxicity was only observed with peripheral blood mononuclear cells (CC50 of 0.5 ± 0.3 mg/mL) the extract did not affect the proliferative status of human lymphocytes (Haasbach *et al.*, 2014).

3.3.8. Conclusions

The data on toxicology of *Ribes nigrum* L., folium and relevant preparations are limited.

However, the medicinal use of *Ribes nigrum* L., folium is considered safe because no adverse effects have been reported during the long-standing use as a medicinal product in France, Poland and Spain. Due to the lack of adequate data on genotoxicity a list entry cannot be proposed.

3.4. Overall conclusions on non-clinical data

The medicinal use of *Ribes nigrum* L., folium described in several monographs and well-known handbooks as well as of the products available on the EU market for the traditional herbal medicine is supported by the effects observed in the non-clinical (*in vitro, ex vivo* and *in vivo* data) studies.

Based on the available pharmacological data the following uses are plausible

- for the relief of minor articular pain;
- to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

Pharmacological effects not related to the indications proposed for the *Ribes nigrum* L., folium in the monograph are antioxidative effects, antimicrobial, antiviral and antihypertensive effect.

None of the reported non-clinical pharmacological studies described indicate a cause for safety concern for the *Ribes nigrum* L., folium.

The use in the European Community and available data indicate no toxicological concern and potential risks associated with *Ribes nigrum* L., folium use.

As there is no 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 clinical data available.

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

No data available.

4.2. Clinical efficacy

4.2.1. Dose response studies

No dose response studies available.

4.2.2. Clinical studies (case studies and clinical trials)

No clinical studies reported.

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

No clinical studies in special populations reported.

4.4. Overall conclusions on clinical pharmacology and efficacy

For the *Ribes nigrum* L., folium no data from clinical studies are available therefore, in accordance with Directive 2001/83/EC the well-established use cannot be supported.

The traditional herbal medicinal use of *Ribes nigrum* L., folium for the indications which are proposed in the monograph is supported by products in the EU market, *in vitro* and *in vivo* pharmacological results and information available in several monographs, pharmacopoeias and well-known handbooks.

5. Clinical Safety/Pharmacovigilance

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

No data collected.

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

No data available.

5.4. Laboratory findings

None reported.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

The use in children and adolescents has not been investigated and is not supported by the traditional use. Therefore, the use in children and adolescents under 18 years of age is not recommended.

5.5.2. Contraindications

Oedema due to heart failure or renal insufficiency is mentioned as a possible contra-indication without any further specification (Hänsel *et al.*, 1994; Gruenwald *et al*., 2000).

The monograph includes a contraindication for persons with hypersensitivity to the active substance and in conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal disease).

5.5.3. Special warnings and precautions for use

No data available.

However, to exclude serious diseases and worsening of the complaints the following warnings that are proposed for the monograph:

- if complaints or symptoms such as fever, dysuria, spasms or blood in the urine occur during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted;
- concomitant treatment with synthetic diuretics is not recommended;
- articular pain accompanied by swelling of joints, redness or fever, should also be examined by a doctor.

5.5.4. Drug interactions and other forms of interaction

No data available.

5.5.5. Fertility, pregnancy and lactation

No data is available, therefore, use of *Ribes nigrum* L., folium cannot be recommended during pregnancy and lactation.

5.5.6. Overdose

No data available.

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

No data available.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

For *Ribes nigrum* L., folium there are no clinical safety data available. Also, the use in children or adolescents is not documented in literature.

The available information in the literature and pharmacoviligance data of marketed products do not indicate safety concerns for *Ribes nigrum* L., folium. As a precautionary measure a contraindication and warnings are included in the monograph.

6. Overall conclusions (benefit-risk assessment)

Pharmacological data substantiate the traditional use of blackcurrant leaves in inflammatory conditions and on the urinary tract. There is also experimental evidence for analgesic activity. To date, no general or gastro-intestinal toxicity has been reported.
The pathological conditions for which Ribis nigri folium is used are mostly symptomatically approached. Due to the lack of data from clinical studies, well-established use for *Ribes nigrum* L., folium in accordance with Directive 2001/83/EC, cannot be recommended.

There are no published reports on serious side effects when taking therapeutic doses or after (in) voluntary intoxications with the herbal substance or herbal preparations thereof. Secondary metabolites of Ribis nigri folium have a polyphenolic character and can be considered as antioxidants.

There are no concerns of possible drug interactions.

Herbal preparations of Ribis nigri folium have been on the market for more than 30 years in some European countries.

No data on fertility, reproductive and developmental toxicity and the usage in children and adolescents is available. Therefore, the use is not recommended in children, adolescents and during pregnancy and lactation.

Available acute or chronic preclinical toxicity. A European Union list entry is not supported due to lack of adequate data on genotoxicity.

The traditional medicinal use of *Ribes nigrum* L., folium according to Directive 2004/24/EC is fulfilled based on the information available several pharmacopeias, relevant medicinal handbooks, and the information provided by the National Competent Authorities. The available date substantiates the presence of medicinal products on the European market throughout a period more than 30 years, including at least 15 years within the EU.

The traditional use of *Ribes nigrum* L., folium listed in the European Union Community Monograph is plausible on the basis of long-standing use and the evidence of non-clinical data for the following indications:

For oral use:

a) 'Traditional herbal medicinal product for relief of minor articular pain'

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

In the 2017 revision of the monograph, a powdered herbal preparation was added because this product is on the market in France for more than 30 years.

The indications mentioned in the European Union Community Monograph fulfil the requirements for traditional use in that they are suitable for self-medication.

The therapeutic areas for browse search on the EMA website are 'Pain and inflamation' and 'Urinary tract and gynaecology disorders'.

No constituents with known therapeutic activity or active markers could be identified by the HMPC.

Annex

List of references