EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Arctostaphylos uva-ursi* (L.) Spreng., folium

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Arctostaphylos uva-ursi* (L.) Spreng., folium (bearberry leaf)
Herbal preparation(s)	a) Comminuted herbal substance b) Powdered herbal substance c) Dry extract (DER 3.5–5.5:1), extraction solvent ethanol 60% (V/V) containing 23.5–29.3% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry) d) Dry extract (DER 2.5–4.5:1), extraction solvent water containing 20–28% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry) e) Liquid extract (DER 1:1), extraction solvent ethanol 25% V/V
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use Herbal preparations in liquid or solid dosage forms for oral use
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1. Introduction

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

- Herbal substance(s)

Bearberry leaf (Uvae ursi folium) consists of whole or cut, dried leaf of *Arctostaphylos uva-ursi* (L.) Spreng. It contains not less than 7% of anhydrous arbutin ($C_{12}H_{16}O_7$; M_r 272.3), calculated with reference to anhydrous drug determined using HPLC method.

The leaf, shiny and dark green on the adaxial surface, lighter on the abaxial surface, is generally 7-30 mm long and 5-12 mm wide. The entire leaf is obovate with smooth margins, somewhat reflexed downwards, narrowing at the base into a short petiole. The leaf is obtuse or retuse at its apex. The lamina is thick and coriaceous. The venation, pinnate and finely reticulate, is clearly visible on both surfaces. The adaxial surface is marked with sunken veinlets, giving it a characteristic grainy appearance. Only the young leaf has ciliated margins. Old leaves are glabrous. (Ph.Eur. 1054).

- Herbal preparation(s)

a) Comminuted herbal substance as herbal tea

b) Powdered herbal substance

c) Dry extract (DER 3.5 – 5.5:1), extraction solvent ethanol 60% (V/V) containing 23.5 – 29.3% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry)

d) Dry extract (DER 2.5 – 4.5:1), extraction solvent water containing 20 – 28% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry)

e) Liquid extract (DER 1:1), extraction solvent ethanol 25% V/V

Principal constituents of the herbal substance

(Bradley 1992; ESCOP 2003; British Herbal Pharmacopoeia 1996; Gruenwald *et al.,* 2004; Barnes *et al.*, 2002; Frohne 2004; Hänsel *et al.,* 1993; Britton and Haslam, 1965; Frohne, 1977; Jahodář *et al.,* 1978):

Hydroquinone derivatives: arbutin (hydroquinone-*O*-*β*-D-glucoside) 5 – 16%; the arbutin content is seasonally variable, leaf content over 17% has been reported; methyl arbutin (*O*-methyl hydroquinone-*O*-*β*-D-glucoside) up to 4% according to origin of the drug; galloyl derivatives of arbutin 0.05% (*O*-galloyl hydroquinone-*O*-*β*-D-glucoside, 2´´*O*-galloyl arbutin, 6´´*O*-galloyl arbutin); free hydroquinone usually less than 0.3% and methylhydroquinone

The amount of arbutin and methyl arbutin is related to the photometric method with 4-aminoantipyrin-Emerson reaction, while the currently used HPLC method can give different results.

Polyphenols (tannins): 10 – 20%, gallotannins including penta-*O*-galloyl-β-D-glucose and hexa-O-galloyl-β-D-glucose, ellagictannine corilagin (1-*O*-galloyl-3.6-di-*O*-hexahydroxydiphenoyl-*B*-D-glucose), catechin; anthocyanidin derivatives including cyanidin and delphinidin

Phenolic acids: approximately 0.25% in free form, mainly gallic, p-coumaric and syringic acids, but also salicylic acid, p-hydroxybenzoic acid, ferrulic acid, caffeic acid and lithospermic acid (dimeric caffeic acid)

Piceoside: (4-hydroxyacetophenone-O-β-D-glucopyranoside)

Flavonoids: hyperoside (0.8 – 1.5%), quercitrin-3-β-D-O-6´´-galloyl galactoside, quercitrin, isoquercitrin, myricitrin, myricetin-3-O-βD-galactoside, two isomeric quercetin arabinosides, aglycones of these compounds, kaempherol

Iridoid glucoside: monoterpein (0.025%)

Triterpenes: 0.4 – 0.8%, including ursolic acid, uvaol, α-amyrin, α-amyrin acetate, β-amyrin, lupeol, mixture of mono- and di-ketonic α-amyrin derivatives

Enzymes: β-glucosidase (arbutase)

Other constituents: allantoin, resin (e.g. ursone), volatile oil (trace) and wax

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

Not applicable

1.2. Search and assessment methodology

Databases and other sources used to research available pharmaceutical, non-clinical and clinical data on *Arctostaphylos uva-ursi* (L.) Spreng., leaf, or its relevant constituents.

Relevant articles and references retrieved from databases: PubMed, MEDLINE, Embase, Biosis, SciSearch, TOXNET. Search term: *Arctostaphylos*, bearberry leaves, Uvae ursi folium.

Literature was provided by AESGP in response to the call for scientific data in January 2016.

Libraries: EMA library, library of the State Institute of Drug Control, Prague.

Textbooks, pharmacopoeias and monographs.

A literature search was performed in April 2016.

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

According to the information provided by the National Competent Authorities in the overview of the marketed products, the following herbal substances/preparations have been marketed in the EU/EEA.

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form**	**Regulatory Status**
Comminuted herbal substance	Inflammatory diseases of the urinary tract	Herbal tea > 12 years: For drinking: 3 g/150 ml boiling water, brewing time 10-15 min or 3 g/150 ml cold water, brewing time several hours followed by heating up until boiling point Up to 4 times daily No longer than 1 week and maximum 5 times per year without medical advice	Standard Marketing Authorisation according to section 36 of the German Medicinal Products Act since1986 until 2011 Germany 245 herbal teas with Uvae ursi folium as a single active ingredient
	Inflammatory diseases of the urinary tract collection system	Herbal tea 2 sachets each of 1.5 g poured with 150 ml boiling water/15 min up to 4 times daily	WEU At least since 1976 until 06/2011 Germany
	Tea has diuretic and urinary antiseptic properties	Herbal tea prepared from one tablespoon of the herbal substance 5-6 times daily	WEU Since 2005 Estonia
	Inflammatory disorders of the efferent urinary tract	1,5-3 g dried comminuted leaves for preparation of an infusion 3 times daily	TU On the market for more than 30 years Lithuania
	Uncomplicated, mild infections of lower urinary tract	Infusion of the dried leaf corresponding to 400-800 mg of arbutin per day; duration of treatment is limited to 1 week and up to 5 times a year	TU Since 1996 Slovenia
Powdered herbal substance	Inflammatory diseases of the urinary tract collection system	Coated tablet containing 500 mg of the powdered herbal substance 6 tablets maximum 4 times daily	WEU At least since 1976 until 06/2012 Germany

Active substance	Indication	Pharmaceutical form	Regulatory Status
	Traditional herbal medicinal product used for treatment of symptoms of mild recurrent lower urinary tract infections such as burning sensation during urination and/or frequent urination in women, after serious conditions have been excluded by a medical doctor.	Capsule containing 350 mg of the powdered herbal substance/cps 1 capsule 3-4 times daily	Registered according to former registration scheme since Dec 1991 Switched to TU in Jan 2011 Spain
	Traditionally used to promote the renal elimination of water. Traditionally used as an adjuvant to diuretic treatments in benign urinary tract conditions.	Hard capsules containing 350 mg of the powdered herbal substance/cps 2 capsules twice daily, if necessary 5 capsules daily	TU Since 1982 France
Dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (2.5:1), extraction solvent: ethanol 50% (V/V)	Urinal antiseptic in case of cystitis for adult females without any other health issues and who are not pregnant.	Coated tablet containing 500 mg of the extract 2 tablets/4 times daily Maximum 5 days	Bibliographical 13/11/2006 Belgium Replaced with below mentioned TU product in 2017
Dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (3,5–5,5:1) extraction solvent: ethanol 60% V/V	Traditional herbal medicinal product used for treatment of symptoms of mild recurrent lower urinary tract infections such as burning sensation during urination and/or frequent urination in women, after serious conditions have been excluded by a medical doctor.	Coated tablet containing 400 mg of the extract (equivalent to 64-96 mg of arbutin) 2 tablets/3 times daily Maximum 7 days	TU Since 30.11.2015 Belgium
		Coated tablet containing 238.7–297.5 mg of the extract corresponding to 70 mg of hydroquinone derivatives calculated as anhydrous arbutin 2 tablets 3 times daily Duration of use:one week or 4 days if symptoms persist or worsen during the use of the medicinal product	TU Since 11.5.2013 Croatia
		Coated tablets, each containing 265 mg of extract corresponding to 62.3-77.7 mg hydroquinone derivates, calculated as anhydrous arbutin. This amount of dry extract corresponds to approximately 1.2 g dried	TU Since 2014 Sweden

Active substance	Indication	Pharmaceutical form	Regulatory Status
		bearberry leaves *Female adults and elderly:* 2 tablets 3 times daily Duration of use: Not to be used for more than one week. If the symptoms persist for more than 4 days or worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.	
Standardized dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (3.5-5.5:1), extraction solvent: ethanol 60% (V/V)	Inflammatory diseases of the urinary tract	Coated tablet 238.7–297.5 mg dry extract corresponding to 70 mg hydroquinone derivatives calculated as anhydrous arbutin, (photometry Ph. Eur. 1998) >12 years: 2 tablets 3 times daily No longer than 1 week and maximum 5 times per year without medical advice	WEU At least since 1976 Germany
Standardised dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (3-4:1), extraction solvent: water	For supportive treatment of inflammatory diseases of the urinary tract	Coated tablet containing 228–315 mg of the extract corresponding to 63 mg anhydrous arbutin, (HPLC) >12 years: 2-3 tablets 4 times daily No longer than 1 week and maximum 5 times per year without medical advice	WEU At least since 1976 Germany
Standardized dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (2.5-4.5:1), extraction solvent: water	Inflammatory diseases of the urinary tract	Film-coated tablet 425.25–519.75 mg dry extract corresponding to 105 mg hydroquinone derivatives calculated as anhydrous arbutin, (photometry Ph. Eur. 1998) >12 years: 2 tablets 2-4 times daily No longer than 1 week and maximum 5 times per year without medical advice	WEU At least since 1976 Germany
	Uncomplicated infections of the lower urinary tract, when antibiotic treatment is not considered essential	Film coated tablets containing 215 mg dry extract (corresponding to 40 mg of arbutin) >12 years: 4 tablets 3 times daily	TU 2002 – 2012 Poland

Active substance	Indication	Pharmaceutical form	Regulatory Status
Dry extract (3.5–6.0:1), extraction solvent water	Traditionally used to promote the renal elimination of water and as an adjuvant to diuretic treatments in benign urinary tract conditions	Capsules containing 200 mg of dry extract/cps 1 capsule twice daily	TU Since 1992 France
Standardized liquid extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (1:0.54-0.99), extraction solvent: water:calcium oxide (44:1)	For supportive treatment of inflammatory diseases of the urinary tract	Oral liquid 10 g (=9.75 ml) contain 3.9-7.1 g extract corresponding to 0.56 g anhydrous arbutin (HPLC) >12 years: 1.8-3.6 ml (103.5-207 mg anhydrous arbutin) Up to 4 times daily No longer than 1 week and maximum 5 times per year without medical advice	WEU 2007 Germany

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

Combination products which indications are the same or similar to the monocomponent products reported by the Member States:

- Extract of Uvae ursi folium, Millefolii herba, Agrimoniae herba, Urticae folium, Equiseti herba, Betulae folium, extraction solvent 20% ethanol (DER 1:4) – tincture; indication: mild urinary infections, cystitis or inflammations of the lower urinary tract due to sitting on a cold place; HU since 2001, reclassified to TU 2012.
- Sugar coated tablets containing 80 mg of extract (as dry extract) from bearberry leaf (*Arctostaphylos uva-ursi* (L.) Spreng, folium) (5:2) (equivalent to 200 mg Uvae ursi folium), extraction solvent: water; 60 mg Buchu leaf (*Agathosma betulina* (Berg.) Pillans, folium); 23.4 mg of extract (as dry extract) from Buchu leaf (*Agathosma betulina* (Berg.) Pillans, folium) (100:39) (equivalent to 60 mg Buchu leaf), extraction solvent: water; 16.8 mg of extract (as dry extract) from Clivers herb (*Galium aparine* L., herba) (100:28) (equivalent to 60 mg Clivers herb), extraction solvent: water; 12 mg of extract (as dry extract) from couch grass rhizome (*Agropyron repens* Beauvais, herba) (5:1) (equivalent to 60 mg couch grass), extraction solvent: water; 12 mg of extract (as dry extract) from horsetail herb (*Equisetum arvense* L., herba) (5:1) (equivalent to 60 mg horsetail herb), extraction solvent: water; 12 mg of extract (as dry extract) from Shepherd's Purse herb (*Capsella bursa-pastoris* L., herba) (5:1) (equivalent to 60 mg Shepherd's Purse herb), extraction solvent: water in one tablet; indications: a traditional herbal medicinal product used to help flushing of the urinary tract and to assist in minor urinary complaints associated with cystitis in women, based on traditional use only. Contraindications: among others current or previous kidney disease; undesirable effects: Nausea, vomiting, stomach ache has been reported with Uvae ursi folium. The frequency is not known; UK since 04/2013.
- Oral drops containing 715 mg of tincture from fresh bearberry herb (*Arctostaphylos uva-ursi* (L.) Spreng, herba) (1:4), extraction solvent: ethanol 43% m/m; 240 mg of tincture from fresh *Echinacea purpurea* herb (*Echinacea purpurea* (L.) Moench, herba) (1:12), extraction solvent: ethanol 57.3% m/m in 1 ml of the product; indication: a traditional herbal medicinal product used to help relieve minor urinary complaints associated with cystitis in women, such as burning sensation during urination and/or frequent urination, based on traditional use only; UK, since 10/2015.
- Oral liquid containing 0.875 ml of extract (as liquid extract) from couch grass herb (*Agropyron repens* (L) Beauv., herba) (1:1), extraction solvent: water; 0.5 ml of extract (as liquid extract) from Marshmallow root (*Althaea officinalis* L., radix) (1:1), extraction solvent: water; 1.125 ml of (as liquid extract) from Buchu leaf (*Agathosma betulina* (Berg) Pillans, folium) (1:2.5), extraction solvent: ethanol 25% V/V; 0.16 ml of extract (as liquid extract) from bearberry leaf (*Arctostaphylos uva-ursi* (L.) Spreng., folium) (1:1), extraction solvent: water; 0.16 ml of extract (as liquid extract) from Juniper berries (*Juniperus communis* L., galbulus) (1:1), extraction solvent: water; 0.16 ml of extract (as liquid extract) from Clivers herb (*Galium aparine* L. herba) (1:1), extraction solvent: water; indication: a traditional herbal medicinal product used to help flushing of the urinary tract and to assist in minor urinary complaints associated with cystitis in women only, based on traditional use only; contraindications: among others current or previous kidney disease; undesirable effects: nausea, vomiting and stomach ache have been reported with bearberry leaf. The frequency is not known. UK since 03/2013.

Bearberry leaf is a component of many herbal teas marketed in the Member States with the same or similar indication as monocomponent products and the above-mentioned combination products:

- Uvae ursi folium, Equiseti herba, Myrtilli herba, Matricariae flos, Sambuci flos, Solidaginis herba, Thymi herba; indication: An adjuvant for treatment of symptoms of mild lower urinary

tract infections such as burning sensation during urination and frequent urination; Czech Republic, on the market since 1969, switched to TU in 2011, SK since 1969

- Urticae folium, Uvae ursi folium, Betulae folium, Juniperi pseudo-fructus pulvis; indication: used as a diuretic to prevent the formation and facilitate the expulsion of urinary sand and minor stones. May be applied in urinary tract infections with mild symptoms, only if antibiotic treatment is not needed; Hungary 2006
- Betulae folium, Uvae ursi folium, Ononidis radix, Petroselini radix, Polygoni avicularis herba, Sambuci nigrae flos, Urticae herba, Millefolii herba; indication: used as an adjuvant for treatment of symptoms of mild lower urinary tract infections such as burning sensation during urination and frequent urination; Czech Republic, on the market since 1969, switched to TU in 2011
- Betulae folium, Uvae ursi folium, Herniariae herba, Menthae piperitae herba, Ononidis radix, Petroselini radix; indication: used as an adjuvant for treatment of symptoms of mild lower urinary tract infections such as burning sensation during urination and frequent urination; Czech Republic, on the market since 1995, switched to TU in 2011
- Uvae ursi folium, Betulae folium, Orthosiphonis folium, Solidaginis virgaureae herba; indication: used for flushing of the urinary tract as supportive treatment of recurrent infections after serious conditions have been excluded; Austria – TU since 2012
- Uvae ursi folium, Betulae folium, Orthosiphonis folium, Solidaginis virgaureae herba; indication: used for flushing of the urinary tract as an adjuvant in minor urinary complaints and to decrease sedimentation of renal gravel; Germany, TU since 2013
- Betulae folium, Graminis rhizoma, Solidaginis gig. herba, Ononidis radix, Liquiritiae radix; indication: used for adjuvant treatment in mild inflammation (catarrh) of the bladder and the renal pelvis; DE – Standard Marketing Authorisation (Blasen- und Nierentee II), since 1987
- Uvae ursi folium, Ononidis radix, Orthosiphonis folium, Graminis rhizoma; indication: to increase the amount of urine in inflammation (catarrh) of the lower urinary tract, to prevent formation of renal gravel and uroliths; Germany – Standard Marketing Authorisation (Blasen- und Nierentee IV), since 1988
- Uvae ursi folium, Phaseoli fructus, Solidaginis gig. herba, Orthosiphonis folium; indication: used for adjuvant treatment in mild inflammation (catarrh) of the bladder and the renal pelvis; DE – Standard Marketing Authorisation (Blasen- und Nierentee V), since 1988
- Uvae ursi folium, Betulae folium, Graminis rhizoma; indication: used for adjuvant treatment in mild inflammation (catarrh) of the bladder and the renal pelvis; Germany – Standard Marketing Authorisation (Blasen- und Nierentee VII), since 1988

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

Not available

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

Not available

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

Bearberry leaves use is for the first time literally documented in the Middle Ages in the Welsh "Physicians of Nyddfai" from the 13th century. It seems that bearberry leaf was used in Northern areas as a folk remedy long before it came to the Central Europe. In Renaissance herbaria bearberry was only mentioned occasionally without any link to a specified medicinal use. Bearberry is mentioned for example in "Historia Rariorum Plantarum" by Carolus Clusius, Antwerp (1601) and also by Linné in his

"Materia Medica" from 1749. In Germany, bearberry was used in larger scale since the middle of 18th century. From the beginning of 19th century, bearberry is in official use. It was used for treatment of various diseases such as hydrops, lithiasis, in diabetes, for the therapy of gonorrhoea, etc. Until now only the use as urinary tract antiseptic and diuretic remains. Bearberry leaf was used also in the "New World" by the North American Indians for the treatment of urinary tract diseases (Frohne, 1977).

The medicinal use has been documented continuously in many pharmacopoeias, pharmacognostical texts and handbooks dating e.g. from 1926, 1938, 1947, 1953, 1960, 1977, 1986, 1998, 2002, 2003 and 2009 - Deutsches Arzneibuch DAB 6. Ausgabe (1926), Československý lékopis 1. vydání (1947), Hagers Handbuch der Pharmazeutischen Praxis (Frerichs *et al.,* 1938), Pharmacopoea Helvetica V (1953), Österreichisches Arzneibuch ÖAB 9. Ausgabe (1960), Martindale- The Extra Pharmacopoeia (Wade 1977), Deutsches Arzneibuch DAB 9. Ausgabe (1986), the Complete German Commission E Monographs (Blumenthal *et al.,* 1998), WHO monographs on selected medicinal plants 2002, ESCOP Monographs 2003 and European Pharmacopoeia 9.0 (2017). Bearberry leaf is traditionally used for the treatment of urinary tract disorders.

The following traditional uses and posologies have been recorded for bearberry leaf

The Complete German Commission E Monographs (Blumenthal *et al.,* 1998)
Uses: inflammatory disorders of the efferent urinary tract. Posology: 3 g drug to 150 ml water as an infusion or cold macerate or 100–210 mg hydroquinone derivatives, calculated as water free arbutin up to 4 times daily. Duration of use: Not to be taken for longer than a week or more than five times a year without consulting a physician. Interaction with other drugs: Preparations of bearberry leaf should not be taken together with drugs that cause acidic urine since this reduces the antibacterial action.

WHO Monographs on Selected Medicinal Plants (Volume 2, 2002)
Uses: described in pharmacopoeias and in traditional systems of medicine: as a mild urinary antiseptic for moderate inflammatory conditions of the urinary tract and bladder, such as cystitis, urethritis and dysuria.
Uses: described in folk medicine: as a diuretic, to stimulate uterine contractions, and to treat diabetes, poor eyesight, renal or urinary calculi, rheumatism and venereal disease, topically for skin depigmentation. Posology: 3 g of the drug/150 ml in a form of infusion or cold macerate 3 to 4 times daily; 400 – 840 mg hydroquinone derivatives; other preparations accordingly calculated as arbutin. Duration of use: Not to be used for prolonged period. Patients have been advised to avoid eating highly acidic foods and to drink plenty of fluids.

ESCOP Monographs (2003)
Therapeutic indications: uncomplicated infections of the lower urinary tract such as cystitis, when antibiotic treatment is not considered essential. Posology: cold water infusions of the dried leaf corresponding to 400 – 800 mg of arbutin per day, divided into 2 to 3 doses; equivalent preparations; not recommended for children. Duration of use: treatment could be continued until complete disappearance of symptoms (up to maximum of 2 weeks); if symptoms worsen during the first week of treatment medical advice should be sought. Patients should be advised to consume plenty of liquid during the treatment; alkalisation of the urine may be beneficial. Interactions with other drugs: concomitant acidification of the urine (by other remedies, for instance) may result in a reduction of efficacy

British Herbal Pharmacopoeia (1983)
Indications: acute catarrhal cystitis with dysuria and highly acid urine
Posology: dried leaves 1.5 – 4 g three times daily or by infusion; concentrated infusion (BPC 1934) 2–4 ml three times daily; liquid extract 1:1 in 25% ethanol 1.5 – 4 ml three times daily

British Herbal Compendium (Bradley, 1992), *British Herbal Pharmacopoeia* (1996)

Indications: mild infections of the urinary tract. Posology: three to four times daily 1.5 – 2.5 g dried leaf, in infusion or cold aqueous extract; liquid extract (1:1) extraction solvent ethanol 25% V/V 1.5 – 2.5 ml; tincture (1:5), extraction solvent ethanol 25% V/V 2 – 4 ml. Duration of use: short treatment (maximum of 7 days). An “alkaline„ diet, high vegetables and fruit, should be taken during treatment.

Herbal Medicines. A guide for healthcare professionals (Barnes *et al.,* 2002)
Barberry is a diuretic and astringent and has been stated to exert an antiseptic effect on the urinary tract. Traditionally, it has been for cystitis, urethritis, dysuria, pyelitis, lithuria, and specifically for acute catarrhal cystitis with dysuria and highly acidic urine. Posology: dried leaves 1.5 – 4.0 g as an infusion three times daily; liquid extract (1:1), extraction solvent ethanol 25% 1.5 – 4.0 ml three times daily.

Martindale Extra Pharmacopoeia (Wade, 1977)
Barberry is a diuretic and astringent and has been stated to exert an antiseptic effect on the urinary tract. Posology: fresh infusion (1 in 20) 15–30ml; concentrated infusion (1 in 2.5) 2-4 ml; liquid extract (1:1) 2 ml

PDR for Herbal Medicines (Gruenwald *et al.,* 2004)
Indications: infections of the urinary tract - for inflammatory disorders of the efferent urinary tract Posology: a daily dose of finely cut or powdered drug 10 g (corresponding to 400 – 840 mg of arbutin) or 3 g of the drug/150 ml in form of an infusion or cold macerate up to 4 times daily or 400 – 840 mg hydroquinone derivatives calculated as water-free arbutin; 0.4 g of dry extract in single dose; liquid extract single dose 2 g. The urine should be alkaline.

Standard Zulassungen 1996 (Bärentraubenblätter monograph dated 1986), *Hagers Handbuch der Pharmazeutischen Praxis* (Hänsel *et al*., 1993)
Indications: used as an adjuvant in therapy of bladder and renal pelvis catarrhs. Posology: 1 cup of a decoct prepared from 1 teaspoon (approximately 2 g) of pulverised drug boiled with 150 ml of water for 15 minutes or a macerate prepared with cold water (after several hours maceration) 3 to 4 times daily. Vegetable diet is recommended to achieve alkaline urine; additionally, sodium hydrogen carbonate can be used. Duration of use: not to be used for long time without consultation with a doctor. Interaction with other drugs: should not be taken together with drugs that cause acidic urine.

Martindale, The Extra Pharmacopoeia (2004)
Bearberry has been reported to be a diuretic, bacteriostatic, and astringent and has been used in the treatment of urinary tract disorders.

Hagers Handbuch der Pharmazeutischen Praxis (Kern *et al.,* 1972)
Indications: as a disinfectant in disorders of the urinary tract, particularly in chronic urethral and bladder catarrh. Posology: 1.3 – 4 g of the pulverised drug or as a decoction prepared from 1.5 g of the herbal substance per one cup, daily dose 10 to 15 g.

Český lékopis (2005)
Posology: single dose 3 g, daily dose 12 g. Duration of use: maximum 2 weeks.

Table 2: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Dried herbal substance	Infections of the urinary tract - for inflammatory disorders of the efferent urinary tract	3 g /150 ml as an infusion or cold macerate up to 4 times daily or 400 – 840 mg hydroquinone derivatives calculated as water-free arbutin	Gruenwald *et al.,* 2004
	Inflammatory disorders of the efferent urinary tract	3 g/150 ml of water as an infusion or macerate equivalent to 100 - 210 mg hydroquinone derivatives up to 4 times daily Maximum one week and 5 times per year;not to be used for prolonged period	Blumenthal *et al.,* 1998, WHO monograph 2002
	Uncomplicated infections of the lower urinary tract	cold water infusions of the dried leaf corresponding to 400 – 800 mg of arbutin per day, divided in 2 or 3 doses Maximum 2 weeks	ESCOP, 2003
	Mild infections of the urinary tract	1.5-2.5 g 3-4 times daily or in infusion or cold aqueous extract Maximum one week	Bradley, 1992
	Traditionally used for cystitis, urethritis, dysuria, pyelitis, lithuria, and specifically for acute catarrhal cystitis with dysuria and highly acidic urine	dried leaves 1.5 – 4.0 g as an infusion three times daily	Barnes *et al.,* 2002
	Acute catarrhal cystitis with dysuria and highly acid urine	dried leaves 1.5 – 4 g three times daily or by infusion; concentrated infusion (BPC 1934) 2 – 4 ml three times daily	British Herbal Pharmacopoeia 1983
	As an adjuvant in therapy of bladder and renal pelvis catarrhs	1 cup of a decoct prepared from 1 teaspoon (approximately 2 g) of pulverised drug or a macerate prepared with cold water (after several hours maceration) 3 to 4 times daily not to be used for long time without consultation with a doctor	Standard Zulassungen 1996 (Bärentraubenblätter monograph dated 1986), Hänsel *et al.,* 1993
	As disinfectant in disorders of the urinary tract, particularly in chronic urethral and bladder catarrh	as a decoction prepared from 1.5 g of the herbal substance per one cup, daily dose 10 to 15 g	Kern *et al.,* 1972
Powdered herbal substance	Mild infections of the urinary tract	three to four times daily dried leaf, 1.5 – 2.5 g	Bradley 1992, *British* Herbal Pharmacopoeia 1996
	Infections of the urinary tract - for inflammatory disorders of the efferent urinary tract	a daily dose of 10 g finely cut or powdered drug (corresponding to 400 – 840 mg of arbutin)	Gruenwald *et al.,* 2004
	As an disinfectant in disorders of the urinary tract,	1.3 – 4 g of the pulverised drug, daily dose 10 to 15 g	Kern *et al.,* 1972

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
	particularly in chronic urethral and bladder catarrh		
Liquid extract (1:1), extraction solvent ethanol 25% (V/V)	Mild urinary tract infections	1.5-4.0 ml three times daily	Bradley, 1992, Barnes *et al.*, 2002. British Herbal Pharmacopoeia 1983
Tincture (1:5), extraction solvent ethanol 25% (V/V)	Mild urinary tract infections	2-4 ml three times daily Maximum one week	Bradley, 1992, Barnes *et al.*, 2002, British Herbal Pharmacopoeia 1996

There is different information on tea preparation in different literature sources. Herbal tea could be prepared by decoction from the powdered herbal substance (DAB 9, Blumenthal *et al.,* 1998; Standard Zulassungen 1996; Weiss, 1985) or as an infusion from cut or powdered herbal substance (Blumenthal *et al.,* 1998; Gruenwald *et al.,* 2004) or by maceration for several hours (Standard Zulassungen, 1996; Gruenwald *et al.,* 2004). Results of the research done by Frohne (1970) are summarised in table 3:

Table 3

Droge	Folia uvae ursi, Handelsdroge					Teemischungen (species urologicae)	
Zerkleinerungsform:	minutim concis	minutim concis	plv. gross.	minutim concis	plv. gross.	A) concis-Drogen	B) Ganzdrogen
Ansatzverhältnis (Droge/Wasser)	10 g/150 ml	10 g/150 ml	10 g/150 ml	2 g/150 ml	2 g/150 ml	6 g/150 ml*	6 g/150 ml*
Zubereitung:	mit kochendem Wasser übergießen, 10 Min. ziehenlassen	15 Min. kochen	30 Min. kalt extrahieren (Schüttelmaschine)	15 Min. kochen	30 Min. kalt extrahieren (Schüttelmaschine)	mit kochendem Wasser übergießen; 10 Min. ziehenlassen	
Extraktmenge:	1) 131,5 ml 2) 129 ml	1) 107 ml 2) 122 ml	1) 112 ml 2) 120 ml	1) 142 ml 2) 146 ml	1) 141 ml 2) 141 ml	1) 120 ml 2) 115 ml	1) 127 ml 2) 120 ml
Arbutinkonkonzentration	1) 0,32% 2) 0,35%	1) 0,59% 2) 0,56%	1) 0,71% 2) 0,72%	1) 0,11% 2) 0,12%	1) 0,13% 2) 0,13%	1) 0,033% 2) 0,056%	1) 0,005% 2) 0,009%
Arbutinmenge pro Tasse	1) 422 mg 2) 447 mg	1) 634 mg 2) 683 mg	1) 800 mg 2) 864 mg	1) 162 mg 2) 168 mg	1) 189 mg 2) 189 mg	1) 38 mg 2) 60 mg	1) 4 mg 2) 7,6 mg

Alle Bestimmungen kolorimetrisch mit 4-Aminoantipyrin

* = 1 Eßlöffel pro Tasse Wasser

The content of tannins has not been taken into consideration by Frohne (1970).

During decoction, high amount of tannins is extracted while cold maceration prevents tannins elution. Taking in consideration that, in most literature sources, there is a recommendation to use the comminuted herbal substance in a form of cold macerate or infusion, and the fact that the adverse reactions (gastrointestinal complaints) are in literature attributed to tannins content (Frohne, 2004; Hänsel *et al.,* 1993; ESCOP, 2003), the following instruction is recommended:

To make an herbal infusion, pour 150 ml of boiling water over 1.5 – 4 g of comminuted herbal substance and steep for 10 to 15 minutes.

To make a macerate, pour 150 ml of cold water over 1.5 – 4 g of the comminuted herbal substance and steep for minimum 30 minutes stirring frequently. The macerate should be used immediately after preparation.

2.3. Overall conclusions on medicinal use

Traditional medicinal use of *Arctostaphylos uva-ursi* (L.) Spreng. leaf, is well documented in a number of literature sources. Herbal substance in a form of herbal tea or in powdered form and aqueous and ethanol extracts of the herbal substance are used in the Member States for at least 30 years. Based on information provided by the National Competent Authorities in the overview of the marketed products and literature data the following herbal preparations fulfil the criteria set in Directive 2001/83/EC for at least 30 years of the medicinal use:

Table 4: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Comminuted herbal substance	Inflammatory disorders of the efferent urinary tract	1,5-3 g dried comminuted leaves for preparation of an infusion 3 times daily	On the market for more than 30 years Lithuania
	Acute catarrhal cystitis with dysuria and highly acid urine	dried leaves 1.5 – 4 g three times daily or by infusion	British Herbal Pharmacopoeia 1983
	As a disinfectant in disorders of the urinary tract, particularly in chronic urethral and bladder catarrh	as a decoction prepared from 1.5 g of the herbal substance per one cup, daily dose 10 to 15 g	Kern *et al.,* 1972
Powdered herbal substance	Traditionally used to promote the renal elimination of water. Traditionally used as an adjuvant to diuretic treatments in benign urinary tract conditions	Hard capsules containing 350 mg of the powdered herbal substance/cps 2 capsules twice daily, if necessary 5 capsules per day	Since 1982 France
	As a disinfectant in disorders of the urinary tract, particularly in chronic urethral and bladder catarrh	1.3 – 4 g of the pulverised drug, daily dose 10 to 15 g	Kern *et al.,* 1972
Standardised dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (3-4:1), extraction solvent: water	For supportive treatment of inflammatory diseases of the urinary tract	Coated tablet containing 228–315 mg of the extract corresponding to 63 mg anhydrous arbutin (HPLC) >12 years: 2-3 tablets 4 times daily	At least since 1976 Germany
Standardised dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (2.5-4.5:1), extraction solvent: water	Inflammatory diseases of the urinary tract	Film-coated tablet containing 425.25–519.75 mg dry extract corresponding to 105 mg hydroquinone derivatives calculated as anhydrous arbutin, (photometry Ph. Eur.	At least since 1976 Germany

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
		1998) >12 years: 2 tablets 2-4 times daily	
Standardized dry extract from *Arctostaphylos uva-ursi* (L.) Spreng, folium (3.5-5.5:1), extraction solvent:ethanol 60% (V/V)	Inflammatory diseases of the urinary tract	Coated tablet 238.7–297.5 mg dry extract corresponding to 70 mg hydroquinone derivatives calculated as anhydrous arbutin, (photometry Ph. Eur. 1998) >12 years: 2 tablets 3 times daily No longer than 1 week and maximum 5 times per year without medical advice	At least since 1976 Germany
Liquid extract 1:1, extraction solvent ethanol 25% V/V	Mild urinary tract infections	1.5-4.0 ml three times daily	British Herbal Pharmacopoeia 1983

The following indication is proposed for the European Union Monograph:

Traditional herbal medicinal product used for relief of symptoms of mild recurrent lower urinary tract infections such as burning sensation during urination and/or frequent urination in women, after serious conditions have been excluded by a medical doctor.

A daily dose 10 to 15 g of comminuted and powdered herbal substance is reported in several literature sources. The HMPC decided to keep for the comminuted herbal substance the daily dose of 8 g as approved in the previous version of the monograph and for powdered herbal substance to use the posology from the only marketed French product for which 30 years of medicinal use have been demonstrated. In addition, it is considered not advisable to increase the daily dose up to 15 g per day due to the fact that bearberry leaves contain relatively high amount of tannins, which are reported as responsible for gastrointestinal undesirable effects of bearberry leaf products (Frohne, 2004; Hänsel *et al.,* 1993; ESCOP, 2003). The daily dose of liquid extract (DER 1:1), extraction solvent ethanol 25% (V/V) has been adapted to maximum 8 ml to be in line with the posology for comminuted herbal substance from the same reason as described above.

Two water extracts with DER 3-4:1 and 2.5-4.5:1 authorised in Germany since 1976 were combined in the European Union monograph to “Dry extract (DER 2.5 - 4.5:1), extraction solvent water, containing 20 - 28% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry)”.
Based on the literature data and information received from the Member States and conclusions above, the following posologies are suggested:

Comminuted herbal substance
Female adults and elderly: 1.5-4 g of the comminuted herbal substance in 150 ml of boiling water as a herbal infusion or a macerate, 2 to 4 times daily corresponding to the maximum daily dose of 8 g.

Powdered herbal substance
Female adults and elderly: 700 - 1050 mg twice daily, the maximum daily dose 1.75 g
Dry extract (DER 3.5 - 5.5:1), extraction solvent ethanol 60% (V/V), containing 23.5 - 29.3% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry)
and

Dry extract (DER 2.5 - 4.5:1), extraction solvent water, containing 20 - 28% of hydroquinone derivatives calculated as anhydrous arbutin (spectrophotometry)
Female adults and elderly: Single dose corresponding to 100-210 mg of hydroquinone derivatives calculated as anhydrous arbutin, 2 to 4 times daily. The daily dose is 200 – 840 mg.

Liquid extract (DER 1:1), extraction solvent ethanol 25% (V/V)
Female adults and elderly: 1.5 – 4 ml up to three times daily, the maximum daily dose 8 ml.

3. Non-Clinical Data

Arctostaphylos uva-ursi and its leaf preparations are generally considered to have antibacterial activity and are traditionally used for treatment of the lower urinary tract infections. Published literature provides information on antibacterial activity of bearberry leaf preparations together with several other activities. Publicly available is also information on arbutin and hydroquinone which are the components generally considered to be responsible for the antibacterial activity of the extract. (Gruenwald *et al.,* 2004, Kedzia *et al.,* 1975, Jahodář *et al.*, 1983).

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

3.1.1. Primary pharmacodynamics

Bearberry leaf extract

Antimicrobial effect

In vitro

A bearberry leaf extract (liquid extract 1:5, extraction solvent ethanol 70%) exhibited antimicrobial activity towards a variety of organisms including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Mycobacterium smegmatis, Shigella sonnei* and *Shigella flexneri* (Moskalenko, 1986).

A decoction of bearberry leaf (10 g/100 ml water) increased remarkably the hydrophobicity of both *E. coli* and *Acinetobacter baumannii* strains. There was no growth registered after the exposure of 20 different *E. coli* strains to the undiluted decoction of bearberry and in case of two-fold dilution only one strain showed growth afterwards in peptone broth. This antimicrobial activity could be connected to the ability of bearberry leaf aqueous extract to increase aggregation of Gram-negative bacteria mainly caused by increased hydrophobicity of their cell surface (Türi *et al.,* 1997). A bearberry leaf aqueous extract (decoction 1:10/30 min/100°C/pH 4.7) exhibited a similar effect on *Helicobacter pylori* (Annuk *et al.,* 1999).

The antimicrobial activity of an ethanol extract of the aerial parts of *Arctostaphylos uva-ursi* (prepared by maceration with ethanol 80% for 4 days, ratio solvent to plant material 5:1) and its ethyl acetate fraction (10 ml of the ethanol extract above was evaporated to remove ethanol, dry residue was diluted with water 1:1 and extracted with ethyl acetate, then the solvent was evaporated to dryness) was tested *in vitro* against *E. coli*, *Proteus vulgaris*, *Streptococcus faecalis* and *Enterobacter aerogenes*. Bearberry ethanol extract and its ethyl acetate fraction showed antimicrobial activity against all the microorganisms tested. The inhibitory activity was compared to the antimicrobial activity of streptomycin. The highest activity found in the experiment was approximately 1/500 of the activity of streptomycin against *E. coli,* 1/600 against *P. vulgaris* and 1/100 against *Enterobacter aerogenes* for ethanol extract and 1/100 of the activity of streptomycin against *E. coli,* 1/300 against *P. vulgaris* and 1/800 against *Enterobacter aerogenes* for ethyl acetate fraction. In case of *Streptococcus faecalis*, the

activity corresponded to the activity of streptomycin; however, streptomycin is known to be less active against these bacteria (Holopainen *et al.,* 1988).

Aqueous and methanolic (extracts of bearberry leaves (5 g of the pulverised drug extracted with 100 ml of the solvent, at the end lyophilised; test solution 20 mg/ml) inhibited the growth of *Streptococcus mutans* OMZ176 *in vitro* (Namba *et al.,* 1981).

A 30% ethanol extract of Uvae ursi folium inhibited the growth *in vitro* of *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens* and *Staphylococcus aureus* (Leslie GB, Medita 1978 in WHO 2002).

Ethanol extract (no details on ethanol concentration and DER) was demonstrated not active against *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *E. coli* (Gottshall *et al.,* 1949).

A methanol extract (5 g of the drug macerated in 25 ml of methanol for 24 hours and subsequently dried) has been proved active against *Klebsiella pneumoniae*, *Candida albicans* and *Mycobacterium phlei* at MIC (minimal inhibitory concentration) 4 g/l and against *Staphylococcus aureus* at MIC 2 g/l related to the dried plant, while a chloroform extract prepared by the same way was demonstrated inactive (Ríos *et al.,* 1987).

A bearberry leaf extract (95% ethanol extract – herbal substance to solvent ratio 15:100) alone displayed no antimicrobial activity against any of the 25 bacteria tested (Dykes *et al.,* 2003).

A bearberry aqueous extract (decoction 1:10/30 minutes at 100 °C; tannins content 19.2 mg/ml) exhibited activity to decrease cell surface hydrophobicity (CSH) as well as antibacterial activity against *Helicobacter pylori*. As tannic acid tested in parallel to bearberry leaf water extract exhibited similar results it is suggested by the author that tannic acid may be the component of the extract with the strongest effect in relation the CSH of *Helicobacter pylori* (Annuk *et al.,* 1999).

Different extracts of bearberry leave (aqueous, ethanol and ethyl acetate extracts prepared from 10 g of plant material and 600 ml of the solvent in three equal portions of 200 ml and subsequently evaporated to dryness) were tested for their antimicrobial activity against ten *Enterococcus faecalis* and *E. coli* strains. The aqueous extract showed stronger antibacterial effect on *E. coli* than the other tested extracts, while effect on *Enterococcus faecalis* were similar for all three extracts. Generally, extracts exhibited stronger antibacterial activity against Gram-positive strains (Vučić *et al.,* 2013).

Aqueous and ethanol extracts from *Arctostaphylos uva-ursi* leaves were tested for antibacterial and quorum sensing regulatory activities. The powdered plant material was extracted with hot water (6 g of the drug/50 ml and boiled for 15 minutes) and with ethanol 45% V/V (3 g/25 ml macerated for 24 hours). The liquid extracts were dried. From the dry extracts, hydrophobic fraction was isolated and used for bioactivity analyses using two strains of *Chromobacterium violaceum*. *Chromobacterium violaceum* is well known for its resistance against numerous antibiotics and drug efflux pump genes and thus represents a model for antibacterial screening. Three types of activity and their combinations were revealed; direct antimicrobial activity, non-specific and specific pro /QS (quorum sensing) activities and anti-QS activity. Both extracts led to strong growth and pigment inhibition in both bacterial strains (Tolmacheva *et al.,* 2014).

An ethanol extract from *Arctostaphylos uva-ursi* leaves (prepared by extraction of 5 g of the powdered drug with 25 ml of 55% ethanol V/V for 12 to 18 hours and subsequently freeze dried) was demonstrated effective against *Neisseria gonorrhoeae* isolates at MIC 32 µg/ml (Cybulska *et al.,* 2011).

Diuretic effect

In vivo

In an experimental study diuretic effect of several flavonoid drugs including Uvae ursi folium was investigated. The following preparations were administered to dogs: the flavonoid fraction isolated from the crude drug, a dry methanol extract, a dry aqueous extract, a decoction and an aqueous suspension of the pulverised drug (no details on preparations are available). The starting herbal substance contained 1.5% of total flavonoids. Preparations of Uvae ursi folium inhibited diuresis (Borkowski, 1960 – abstract).

Additionally, no diuretic effect of Uvae ursi tea is reported by Weiss (1985). However, the author did not provide any references supporting this statement.

Contrary to the above-mentioned references diuretic effect of bearberry leaf extracts has been demonstrated in other studies. An aqueous extract of bearberry leaves (no other information on the type of extract and DER) was administered intraperitoneally (i.p.) to 10 male rats as a single dose of 50 mg/kg body weight; a control group of 10 rats received hypotonic saline solution and another group of 10 rats received the diuretic compound hydrochlorothiazide at 10 mg/kg body weight. The urine volume from rats treated with the extract was significantly higher ($p<0.001$ from the 4^{th} to 8^{th} hour after administration; $p<0.05$ over a 24-hour period) than that from the controls and was comparable to the volume after hydrochlorothiazide treatment. No increase in sodium and potassium excretion was observed after administration of bearberry leaf extract (Beaux *et al.,* 1999).

Diuretic effect of crude aqueous extract of *Arctostaphylos uva-ursi* (no information on plant part used, type of the extract and DER) was evaluated after oral administration to mice at the dose of 300 mg/kg. The diuretic effect was assessed by measuring urine volume in ml in comparison with the standard (furosemide 10 mg/kg) and control (not specified). Diuretic activity of Uvae ursi crude extract (2.65±0.0033 ml) was after 4 hours even higher than activity of furosemide (about 2.5 ml) (Saeed *et al.,* 2015).

Arbutin and hydroquinone

Antimicrobial effect

In vitro

The antimicrobial activity of arbutin towards bacteria implicated in urinary tract infections was found to be directly dependent on the β-glucosidase activity of the infective organism. The highest enzymatic activity was shown by *Streptococcus faecalis*, *Klebsiella* and *Enterobacter* genera and by *Proteus vulgaris* and the lowest by *E. coli*. The minimum inhibitory concentration of arbutin is reported to be 0.4 – 0.8% depending on the microorganism (Jahodář *et al.,* 1985). In summary, it has been suggested that a direct relationship exists between the antibacterial action of arbutin and the degree of enzymatic activity of the microorganism.

Arbutin (at concentration 0,5% m/V) after its hydrolysis to hydroquinone showed inhibition of the growth of *Ureaplasma urealyticum* and *Mycoplasma hominis in vitro* (Robertson and Howard, 1987).

Table 5: 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**
Comparable/similar preparations to preparations of the monograph				
Bearberry leaf liquid extract 1:5, extraction solvent ethanol 70%	400 µg/disc	*In vitro*	Moskalenko 1986	Antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, E. coli, Mycobacterium smegmatis, Shigella sonnei* and *Shigella flexneri*
Decoction of bearberry leaf (10 g/100 ml)	The aqueous extract diluted in peptone broth 1:2, 1.4 and 1:8 To the suspension of microorganisms $3x10^9$/ml the same amount of extract was added to a final concentration of microbes $1.5x10^9$	*In vitro*	Türi *et al.*, 1997	Antimicrobial effect against *E. coli* and *Acinetobacter baumannii* strains
	180 µl of the extract added to 180 µl of bacterial suspension (10^9 cells ml^{-1})	*In vitro*	Annuk *et al.*, 1999	Antimicrobial activity against *Helicobacter pylori*
Aqueous extract of bearberry leaves (no information on type of extract and DER)	Single dose: 50 mg/kg bw Intraperitoneal administration	*In vivo* Diuretic effect on rats	Beaux *et al.*, 1999	Increase of urine volume comparable to the volume after hydrochlorothiazide treatment
Aqueous extract of bearberry leaves (5 g of pulverised drug with 100 ml of water, et the end lyophilised)	Concentration of the solution - 20 mg of lyophilizate/ml	*In vitro*	Namba *et al.*, 1981	Inhibited of growth of *Streptococcus mutans*
Dry aqueous extract, decoction and aqueous suspension of powdered drug containing 1.5% of total flavones of which 1.3% hyperoside	The dose corresponding to 4 mg of hyperosides/kg bw Route of administration not specified	*In vivo* Diuretic effect on dogs	Borkowski 1960 - abstract	Inhibition of diuresis
Dry extracts of bearberry leaves (aqueous, ethanol prepared from 10 g of plant material and	Aqueous extract MIC 0.625 – 10 mg/ml Ethanol extract MIC 5-10 mg/ml	*In vitro*	Vučić *et al.*, 2013	Aqueous extract strong antibacterial effect on *E. coli,* both extracts similar activity *on Enterococcus faecalis*

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
600 ml of the solvent and evaporated to dryness)				
Dry ethanol extract from *Arctostaphylos uva-ursi* leaves (prepared by maceration of 5 g of the powdered drug with 25 ml of 55% ethanol V/V,freeze dried)	MIC 32 µg/ml	*In vitro*	Cybulska *et al.*, 2011	Effective against *Neisseria gonorrhoeae* isolates
Crude aqueous extract of *Arctostaphylos uva-ursi* (no information on plant part used, type of the extract and DER)	300 mg/kg oral administration	*In vivo* Diuretic effect on mice	Saeed *et al.*, 2015	Diuretic activity higher than activity of furosemide
Other preparations				
Hydrophobic fraction isolated from dry water and ethanolic extract prepared as a decoction from 6 g of the drug/50 ml, resp. macerate from 3 g of the drug and 25 ml of ethanol 45% V/V and then dried	300 µl/plate	*In vitro*	Tolmacheva *et al.*, 2014	Strong growth and pigment inhibition in *Chromobacterium violaceum* strains
Extract of bearberry leaves, extraction solvent methanol and methanol 50%, (5 g of pulverised drug with 100 ml of solvent, et the end lyophilised)	Concentration of the solution - 20 mg of lyophilizate/ml	*In vitro*	Namba *et al.*, 1981	Inhibited of growth of *Streptococcus mutans*
Extract of bearberry leaf, extraction solvent ethanol 95%, ratio solvent:plant material 100:15	2 µl of the solution with concentration 5000 µg/ml in 3 ml of liquid media	*In vitro*	Dykes *et al.*, 2003	No antimicrobial activity against any of the 25 bacteria tested
Dry ethyl acetate extract prepared from 10 g of plant material and 600 ml of solvent and evaporated to dryness)	MIC 10 mg/ml	*In vitro*	Vučić *et al.*, 2013	Similar inhibition activity on *Enterococcus faecalis* as aqueous extract prepared by the same way

Herbal preparation tested	Strength Dosage Route of administration	Experimental model *In vivo / In vitro*	Reference Year of publication	Main non-clinical conclusions
Single substances				
Arbutin	Arbutin concentrations used 0.08, 0.16, 0.24, 0.32, 0.40, 0.56 and 0.80%	*In vitro*	Jahodář *et al.,* 1985	Inhibited growth of *Streptococcus faecalis*, *Klebsiella* and *Enterobacter* genera, *Proteus vulgaris* and *E. coli*
Arbutin after hydrolysis to hydroquinone	Arbutin concentration 0.5% (m/V)	*In vitro*	Robertson and Howard 1987	Inhibition of the growth of *Ureaplasma urealyticum* and *Mycoplasma hominis*

3.1.2. Secondary pharmacodynamics

Bearberry leaf extract

Anti-urolithic activity

In vitro

Anti-urolithic activity of a crude aqueous extract of *Arctostaphylos uva-ursi* (no information on plant part used, type of the extract and DER) was studied in a test on precipitation of calcium oxalate added to the artificial urine at 37 °C and pH 6.8. Precipitation was measured as turbidity at 620 nm. The time dependent effects of turbidity changes in oxalate solution were tested in artificial urine alone and in combination with extract at concentrations 50, 100, 150, 200 and 250 µg/ml (1ml of the extract solution was added to 0.5 of artificial urine). Inhibition of precipitation/crystallisation was expressed as a percentage of calcium oxalate crystals in presence of extract and without it. The inhibitory activity of the bearberry extract was 95.7% (Saeed *et al.,* 2015).

Anti-inflammatory activity

In vivo

The effect of a 50% methanolic extract from bearberry leaf on the immuno-inflammatory response was studied in contact dermatitis triggered by picryl chloride in mice. When given orally immediately before and 16 hours after the application of picryl chloride, an inhibitory effect on the swelling was observed. A significant therapeutic effect at a dose of 100 mg/kg or more has been demonstrated 24 hours after application (Kubo *et al.,* 1990 – abstract).

Other activities

In vitro

An aqueous extract of the leaves (prepared from 1 part of the herbal substance and 10 parts of water) had antiviral activity *in vitro* against *Herpes simplex* virus type 2, influenza virus A2 (Mannheim 57) and vaccinia virus at a concentration of 10% (May and Willuhn, 1978; WHO, 2002).
The effect of a 50% methanolic extract from bearberry leaf on melanin synthesis was investigated *in vitro*. Bearberry leaf extract as well as arbutin isolated from bearberry leaves had an inhibitory effect on the tyrosinase activity. Furthermore, bearberry leaf extract inhibited the production of melanin from DOPA by tyrosinase and from dopachrome by autoxidation (Matsuda *et al.,* 1992a – abstract).

Water extracts (infusions) from a group of medicinal plants were studied in terms of their activity enhancing the uterine tonus in a series of experiments with a preparation of an isolated rabbit and guinea pig uterine horn. Infusion of bearberry leaves did not show any uterotonic effect (Shipochliev, 1981).

In vivo

Addition of an infusion of the leaves to the drinking-water (3 g/l) of rats fed a standard diet fortified with calcium (8 g/kg body weight) had no effect on urinary calcium excretion and diuresis (Grases *et al.,* 1994).

Arbutin

In vivo

In mice orally or i.p. treated with arbutin (50-200 mg/kg body weight [0.18 – 0.735 mmol/kg]), a dose-dependent antitussive effect to ammonia-induced cough was observed. The antitussive effect of arbutin (200 mg/kg [0.735 mmol/kg]) was as potent as that of codeine phosphate (30 mg/kg), but arbutin had no analgesic or anaesthetic effects. Additionally, arbutin had no effect on tracheal smooth muscle contraction, respiratory activity, spontaneous behaviour, blood pressure, heart rate or electrical activity (Li *et al.,* 1982; NTP 2006).
Male and female cats (none anaesthetised) were administered oral (p.o.) and intraperitoneal (i.p.) doses of 50 and 100 mg/kg [0.18 or 0.367 mmol/kg] body weight arbutin in water and observed at 0.5-, 1-, 2-, and 5-hour intervals. Arbutin at 50 mg/kg body weight (i.p. and p.o.) caused a statistically significant decrease in the number, intensity and frequency of coughs. Similar results were recorded for the 100 mg/kg body weight i.p. and p.o. doses; no increase of the antitussive activity was observed at this dose (Strapkova *et al.,* 1991).

In vitro

Arbutin (in concentrations 90 μg/ml and 40 μg/ml) did not inhibit the growth of rat hepatoma cells (Assaf *et al.,* 1987).

Potential of arbutin to protect radiation-induced apoptosis in human lymphoma U937 cells was evaluated by measuring intracellular hydroxyl radical scavenging ability in these cells. Data in this study indicate that arbutin plays an anti-apoptotic role via decreasing intracellular hydroxyl radical production, inhibition of Bax-mitochondria pathway and activation of the JNK (c-Jun NH(2)-terminal protein Kinase)/p38 MAPK (Mitogen-Activated Protein Kinase) pathway (Wu *et al.,* 2014 – abstract).
An evidence supporting the potential beneficial effect of arbutin alone or in combination with carvedilol in diminishing tissue damage by decreasing phospholipase D, myeloperoxidase and elastase activity and by attenuating the generation of superoxide and subsequently derived reactive oxygen species was provided in the study. The presented data indicated the ability of arbutin to suppress the onset and progression of inflammation (Pečivová *et al.,* 2014).

Effects of arbutin on TCCSUP human bladder carcinoma cell proliferation has been tested by Li *et al.,* 2011. Arbutin did not exhibit any cytotoxic effects in TCCSUP cells at concentrations < 500 μg/ml. To determine the effects of arbutin on cell proliferation, TCCSUP cells were treated with arbutin at various concentrations, and the cell proliferation was measured using the MTT assay. Arbutin significantly decreased TCCSUP cell proliferation in concentration- and time-dependent manner. Furthermore, cell cycle analysis revealed that arbutin strongly disrupted the cell cycle in a time-dependent manner. Western blot analysis demonstrated that arbutin led to the inactivation of extracellular signal-regulated kinase (ERK), which is known to critically regulate cell proliferation. In addition, arbutin markedly increased the expression of $p21^{WAF1/CIP1}$ (p21) which is known to be highly involved in cell cycle

regulation. Results of the study suggest that arbutin inhibits TCCSUP cell proliferation via ERK inactivation and p21 up-regulation (Li *et al.,* 2011).
There are study results providing information that arbutin (2.5, 12.5, or 50 µg/ml [9.2, 45.9, 180 µM]) incubated for 4 days weakly inhibited the growth of human colon carcinoma HCT-15 cells (Kamei *et al.,* 1998; NTP 2006).

3.1.3. Safety pharmacology

Bearberry leaf extract

Safety profile of crude extract of bearberry leaves in rabbits has been investigated. Dry ethanolic extract (DER 11:1; extraction solvent ethanol, concentration not specified) was administered orally for 90 days in male and female rabbits and haematology, biochemistry parameters and histopathology changes were analysed after 90 days. In results of it gender-based variations were observed in haematological, kidney function, liver function, cardiac enzymes and lipids profile. Urine samples revealed the same results as those of standard and control drug. No significant pathology was observed in heart, stomach, liver and kidney tissues of rabbits, treated with bearberry extract in a dose of 25 mg/kg per day (Saeed *et al.,* 2014).

Hydroquinone

Effect of hydroquinone on liver has been studied in a study with rats exposed to 25 and 100 mg/kg body weight of hydroquinone per day for 13 weeks. No hepatotoxicity was observed (Williams *et al.,* 2007, Garcia de Arriba *et al.,* 2013).
In experimental studies, CNS symptoms were observed at hydroquinone oral doses close to lethal dose of 50% (LD50). Repeat dosing in rat and mouse studies caused tremors and reduced activity at doses ≥ 64 mg/kg and convulsions at doses ≥400 mg/kg. These effects were reversible when exposure was discontinued (NTP 1989, 2006, 2009, IARC 1999). Topping *et al.*, 2007 found that tremors occurred within 1 hour following dosing of 64 to 200 mg/kg bw per day to female and male rats for 13 weeks without neuropathological changes.

An NOEL for all CNS effects was experimentally estimated at 20 mg/kg bw per day (IPCS1994, 1996, OECD/SIDS 2002, Garcia de Arriba *et al.,* 2013).

Hydroquinone nephrotoxicity has been linked to the presence of hydroquinone-glutathione conjugates (McGregor 2007) which have been detected in animals after intraperitoneal (i.p.)/subcutaneous (sc) application of free hydroquinone and in much lower degree (4% of de given dose) after oral administration. To date, however, the presence of hydroquinone-glutathione conjugate in plasma/urine of humans has not been reported after oral administration (NTP 2009, DeCaprio1999, Garcia de Arriba *et al.,* 2013).

Hydroquinone administered via gavage for 6 weeks at 50 mg/kg bw to male F344 rats caused proximal tubular damage, as supported by increases in the rate of excretion of renal injury-specific enzymes. Such renal toxicity was not observed in female rats. In this study, no nephrotoxicity was observed in Sprague-Dawley male and female rats (English *et al.,* 1994).

Arbutin

There are no data on safety pharmacology of arbutin available at present.

3.1.4. Pharmacodynamic interactions

Bearberry leaf extract

In mice, *Arctostaphylos uva-ursi* extract or arbutin in combination with prednisolone or dexamethasone inhibited swelling of contact dermatitis induced by picryl chloride (PC-CD) and sheep red blood cell delayed type hypersensitivity (SRBC-DTH) response to a greater extent than either of the two chemicals alone (Kubo *et al.,* 1990 – abstract; Matsuda *et al.,* 1990 – abstract; Matsuda *et al.,* 1991 – abstract).
Water extracts from the leaf of *Arctostaphylos uva-ursi* increased the inhibitory effect of dexamethasone ointment on PC-CD- and carrageenan-induced paw oedema (Matsuda *et al.,* 1992 b – abstract).
An *Arctostaphylos uva-ursi* extract (95% ethanolic extract), enhanced the antimicrobial activity of nisin; bearberry extract alone had no effect (Dykes *et al.,* 2003).

Arbutin

Aloesin (an anti-inflammatory drug) and arbutin synergistically inhibit tyrosinase activity. In a study of their effects on UV-induced pigmentation in human skin *in vivo*, co-treatment with both chemicals (100 mg/g each) produced an additive effect; 63.3% suppression of pigmentation versus 34% with aloesin and 43.5% with arbutin alone (Choi *et al.,* 2002). Additionally, arbutin inhibited UV-induced nuclear factor-kappaB activation in human keratinocytes (Ahn *et al.,* 2003).

Arbutin plus indomethacin showed a stronger inhibitory effect than indomethacin alone in carrageenan-induced oedema and adjuvant-induced arthritis (Matsuda *et al.,* 1991 – abstract).

Arbutin exhibited potent inhibitory effects on rat platelet aggregation induced by adenosine diphosphate (IC_{50}=0.12 mM) and collagen (IC_{50}=0.039 mM) and displayed the same inhibitory activities as the positive control, tetramethylene glutaric acid, on rat lens aldose reductase (Lim *et al.,* 2003 [Korean with English summary]; NTP 2006).

3.1.5. Conclusions

Bearberry leaf extracts and arbutin were tested against several bacterial species, among them bacteria that are representative for uncomplicated urinary tract infections (e.g. *E. coli*, *Shigella* spp, *Streptococcus faecalis*, *Klebsiella* spp*, Enterobacter* spp). It is not clear whether the inhibitory concentrations tested can be reached in human therapeutic conditions.

However, difference in metabolism of arbutin in humans and animals, and metabolism ambiguity of arbutin to hydroquinone should be taken into consideration.

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

In general, non-clinical pharmacokinetic information regarding the crude extract or its components is poor and did not allow any relevant conclusions.

Arbutin, the major constituent of Uvae ursi folium extracts, is a phenolic glycoside, which splits into hydroquinone and glucose (Jahodář *et al.,* 1985). Hydroquinone is the recognised active substance at the site of drug action, which is the lower urine tract. The total amount of hydroquinone (free hydroquinone and hydroquinone conjugates) in urine is crucial for the antimicrobial activity of the herbal preparation. Therefore, human pharmacokinetic studies have been focused on the availability of

total hydroquinone in urine and the use of hydroquinone and arbutin as pharmacokinetic markers is plausible (Paper *et al.,* 1993; Schindler *et al.,* 2002; Quintus *et al.,* 2005).

Bearberry leaf extract

The ability of bearberry leaf to act against urinary infections is believed to be the result of action of free hydroquinone cleaved from the arbutin molecule in the urinary tract. It is not known in which tissue this cleavage occurs *in vivo* and what amount of hydroquinone is present in the organisms after treatment with arbutin. The cleavage is mediated via β-glycosidase, an enzyme which is usually not present in mammalian cells but is present in microorganisms which occur in the gastrointestinal tract or possibly in the urinary tract, when infected (Müller and Kasper, 1996 - abstract).

Five bearberry leaf products (powdered leaves, powdered leaves in capsules, aqueous and methanol extracts; no additional information the extracts) were tested to determine their influence on CYP 3A4, 3A5, 3A7, 2C19 and CYP19-mediated metabolism *in vitro,* as well as on p-glycoprotein efflux activity within human THP-1 and Caco-2 cells. There was difference in the range of inhibition of 3 isozymes of CYP3A family caused by water extract of Uvae ursi folium. The degree of inhibition, from most to least, was in the order CYP3A5 > CYP 3A7 > CYP3A4. Although CYP3A4 was inhibited to a lesser degree than CYP3A5 or CYP3A7, such an inhibitory effect would likely have a greater influence on the elimination of xenobiotics as a result of its biological importance. Methanolic extract inhibited cytochrome P450 enzymes to a lesser extent compared to the water extract. From the pharmacology point of view, the interaction of bearberry leaf extract with CYP isozymes should be carefully considered (Chauhan *et al.,* 2007).

Arbutin

Female Wistar rats were given an aqueous solution of chromatographically pure arbutin, isolated from *Arctostaphylos uva-ursi*, and their excreted urine was evaluated by TLC, HPLC and spectral analysis. Unchanged arbutin was excreted at 82% and 100% after 16 and 30 hours post-treatment, respectively, corresponding to 90.7% of the total arbutin dose administered orally. Arbutin is not changed in urine even at changed pH values. As long as antimicrobial action of arbutin depends on the release of hydroquinone, exo-enzymatic β-glucosidase activity of some microorganisms producing inflammatory processes in the urinary tract must be taken into account (Jahodář *et al.,* 1983).

The highest β–glucosidase extracellular enzymatic activity was found in the genera *Streptococcus faecalis* (100%), *Klebsiella* (95%), and *Enterobacter* (72%), the lowest in *E. coli* (11.6%).
A direct dependence of the antimicrobial effect of arbutin on the level of the enzymatic activity of microorganisms was found. The presumption of the autocidal action of some bacteria on arbutin was confirmed. The minimal bactericidal concentration of arbutin ranges from 0.4 to 0.8%, in dependence on the species of the microorganism (Jahodář *et al.,* 1985).

Oral administration of arbutin (500 mg/kg [1.84 mmol/kg]) to female rats which were overloaded with fluid resulted in a 4-fold excess of excreted urine during the second hour of dosing and a total increase of 61% in the first day. No free hydroquinone was detected in the urine samples. The diuretic activity following treatment with 200 mg/kg hydroquinone was greater than that observed in arbutin-treated animals (NTP 2006).

Investigations were performed in animals to elucidate the absorption, metabolism and elimination of arbutin. Isolated segments of the intestine from the distal part of the duodenum and the caecum of hamster and chicken were used in an *in vitro* model to study in detail the absorption process of arbutin. Experimental data *in vitro* indicate that arbutin is absorbed via the Na^+/glucose carrier in the small intestine. The transport of arbutin in the small intestine was a freely reversible process, also used by glucose and its analogues (Alvarado, 1965; Alvarado and Monreal, 1967).

Arbutin uptake has been also investigated in human small intestine obtained from biopsies of 18 patients with minor abdominal complaints without obvious gastrointestinal disease. In 3 patients, non-tropical sprue was diagnosed. The specimens were differentiated in 4 morphological groups. A significant difference in arbutin uptake between the morphological groups was found. Arbutin uptake was clearly reduced in the case with histological evidence of jejunitis and in cases of non-tropical sprue (Semenza *et al.,* 1969).

Hydroquinone

Studies to determine the absorption, tissue distribution, excretion, and metabolism of [^{14}C]-hydroquinone in male and female rats following single oral, repeated oral, or 24-h dermal administration were conducted by English and Deisinger (2005). Additionally, the concentration of the parent compound in blood following a single 50 mg/kg gavage administration was determined. Absorption into the blood was rapid after oral administration; the maximum concentration was attained within 20 minutes, and the maximum concentration of total ^{14}C within 30 minutes. The parent compound represented ≤1% of total ^{14}C in blood which indicates the extensive first-pass metabolism. Excretion was primarily via urine within 8 hours of gavage. Typically, 87 – 94% of ^{14}C was excreted in urine. Dermal application of ^{14}C-hydroquinone (20µCi) as 5.4% aqueous solution resulted in near background levels of ^{14}C in blood. The major urinary metabolites of hydroquinone were glucuronide and O-sulphate conjugates, which represented 45 – 53 %, and 19 – 33 %, respectively, of an oral dose. A < 5% metabolite was identified as a mercapturic acid conjugate of hydroquinone (English and Deisinger, 2005).
Oral administration of [^{14}C]hydroquinone either in the diet or by gavage to Sprague-Dawley rats results in almost complete absorption from the GI tract, with only about 4% being recovered from faeces. Of the absorbed material, about 91.9% of the radioactivity was recovered from urine (DiVincenzo *et al*, 1984). In a comparison of the kinetics of hydroquinone administered orally and dermally, it was reported that dermal absorption was poor (English and Deisinger, 2005). However, pulmonary absorption, after intratracheal instillation, was very rapid in male Sprague-Dawley rats, with [^{14}C]hydroquinone being detectable in arterial blood within 5 – 10 s. Later blood sampling (45–720 s) indicated rapid metabolism to glucuronides and elimination of the parent compound (Deisinger and English 1999 in McGregor 2007).
Following oral administration to rats, by far the major proportions of metabolites are conjugates of glucuronic (up to 67%) and sulfuric (up to 33%) acids. The remaining urinary metabolites consist of 0 – 5 % mercapturates, 0 – 3% unconjugated hydroquinone and <1% unconjugated 1,4 – benzoquinone (DiVincenzo *et al.,* 1984, English *et al.,* 1988 in McGregor 2007).
The oxidation of hydroquinone to the very reactive 1,4-benzoquinone, which can occur both enzymatically and non-enzymatically, could be of toxicological significance. Hydroquinone auto-oxidises at neutral pH to form 1,4-benzosemiquinone, which can undergo a disproportionation reaction to 1.4-benzoquinone (DiVincenzo *et al.,* 1984; McGregor 2007).

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

In previous sections, it has been addressed that arbutin, a component of bearberry leaf extract is converted to free hydroquinone to exert its antibacterial effect. Based on the pharmacokinetic profile of arbutin, it has been shown that free hydroquinone has an irrelevant accumulation risk since arbutin is very fast conjugated and transformed in innocuous metabolites.

Only in a very low percentage (0.6% of the given dose) free hydroquinone is eliminated via the urine and (<1%) in faeces. Most of the arbutin is therefore transformed in to hydroquinone conjugates (70%) (Siegers *et al.,* 1997; 2003). Free hydroquinone is not accumulated in the animal or human

organism and its potential accumulation is not justified by any pharmacodynamic/pharmacokinetic reason (English and Deisinger, 2005).

The toxicology of free hydroquinone has been reconsidered in several published reviews and it was concluded that there is no evidence to suggest that the toxicity of free hydroquinone is of human relevance (Deisinger *et al.,* 1996; DeCaprio, 1999; McGregor, 2007).

3.3.1. Single dose toxicity

Bearberry leaf extract

No data on single dose toxicity have been reported for bearberry leaf extract.

Arbutin

No data available.

Hydroquinone

The oral LD_{50} of hydroquinone in 2% aqueous solution has been determined as 320 mg/kg in rats, 400 mg/kg in mice, 550 mg/kg in guinea pigs, 300 mg/kg in pigeons, 70 mg/kg in cats and 200 mg/kg in dogs (Woodard *et al.,* 1949).

Acute exposure of rats to high doses of hydroquinone (over 1300 mg/kg body weight) caused severe effects on the central nervous system, including hyperexcitability, tremor, convulsions, coma and death (IPCS 1994).

The presence of food may increase oral LD_{50} values of 310 to 1050 mg/kg in rats. Thus, food showed to decrease the rate and extent of free hydroquinone absorption. LD_{50} values for free hydroquinone by parenteral administration have been reported as 115 – 160 mg/kg in the rat and 190 mg/kg in the mouse (DeCaprio, 1999). In the course of toxicological animal experiments, free hydroquinone demonstrated a very low toxicity since LD_{50} values were very high. In addition, the presence of food importantly improves its safety.

3.3.2. Repeat dose toxicity

Bearberry leaf extract

No data on repeated dose toxicity have been reported for bearberry leaf extract.

Arbutin

Repeated dose toxicity of arbutin has been investigated in mice. At a dose of 8 g/kg [29.38 mmol/kg] administered i.p. for 2 weeks, no toxic effects were observed (Li *et al.,* 1982 [Chinese]; NTP 2006).

3.3.3. Genotoxicity

Bearberry leaf extract

Uvae ursi folium (dry aqueous extract prepared by extraction of 50 g of powdered drug with 300 ml of solvent (40 °C/5 hours) and subsequently dried) has not been mutagenic in the *Salmonella*/microsome assay with *S. typhimurium* strains TA98 or TA100 as well as in the *Bacillus subtilis* rec-assay (Morimoto *et al.,* 1982; WHO 2002).

The cytogenetic effect of ethanolic (70%, DER not specified) extracts of Uvae ursi folium on irradiated human blood lymphocytes was assessed. Micronucleus formation in unirradiated and irradiated

samples of cultured blood lymphocytes using the cytochalasin block micronucleus test was examined. The treatment of cells with bearberry leaf extract did not affect the level of micronuclei in any of the concentration studied (0.025, 0.05, 0.1, or 0.2 mg/ml) (Joksic *et al.,* 2003; NTP 2006).

Arbutin

Arbutin (up to 10^{-2} M [2.73 mg/ml]) did not induce mutations in hamster V79 cells; however, after preincubation of arbutin (≥1 mM [0.3 mg/ml]) with β-glycosidase, it did have a mutagenic effect. An increase in mutation frequency was observed with concentrations of 10^{-3} M arbutin and higher. Hydroquinone, which was used as positive control, exhibited clear effects with a LOEC of about 10^{-5} M. In mice orally treated with arbutin (0.5 – 2 g/kg [2 – 7 mmol/kg] body weight), there was no induction of bone marrow micronuclei. Hydroquinone, which was used as a positive control (50 and 100 mg/kg i.p.) induced clearly elevated micronucleus incidence (Müller and Kasper 1996 – abstract).

The Ames test and the micronucleus test have been performed with urine containing arbutin. Results have shown no indication of genotoxicity related to arbutin (Siegers *et al.,* 1997).

Hydroquinone

Hydroquinone was negative for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of metabolic activation (S9). In Chinese hamster ovary cells, it induced sister chromatid exchanges (with and without S9) and chromosomal aberrations (with S9). Hydroquinone also induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells and was mutagenic in the micronucleus test. Inconclusive results, however, were obtained in *Drosophila* (NTP 1989; NTP 2006). However, hydroquinone was found to be mildly myeloclastogenic in the micronucleus test in SPF mice after oral administration of a toxic dose (200 mg/kg) (Gad-El-Karim *et al.,* 1985).

The DNA reactivity of hydroquinone has been documented in animals and this enhanced activity is presumably due to the oxidised forms of hydroquinone, 1,4-benzosemiquinone and/or 1,4-benzoquinone, which can react with sulfhydryl groups and isolated calf thymus DNA and therefore have the potential to contribute to the toxicity of hydroquinone (McGregor, 2007).

Hydroquinone is generally not active in bacterial tests for mutation, but it has been reported to cause base-pair changes in the oxidant-sensitive strains *Salmonella typhimurium* TA 104 and TA 102, which is consistent with the mutagenicity of 1,4-benzoquinone in several strains of *S. typhimurium*. This result from a single study should not be overemphasised, since it has been suggested that there is little difference in qualitative responses between these two strains and TA 100, against which hydroquinone has been tested to a 13-fold higher dose without any significant response. In other sub mammalian genetic toxicity assays, hydroquinone induced forward mutations but not mitotic recombination or gene conversion in *Saccharomyces cerevisiae*, and it did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* when delivered either in the feed or by injection (McGregor, 2007).

Hydroquinone induced micronuclei and chromosomal aberrations in several studies in bone-marrow cells of mice treated *in vivo*, but not sister chromatid exchanges in a single study. Hyperploidy and chromosome loss (as demonstrated by centromere-positive micronuclei) but not polyploidy was also found in mouse bone marrow. In mouse spermatocytes, chromosomal aberrations and hyperploidy have been observed. In all of these studies *in vivo* dosing was by i.p. injection; however, other dose routes have been used in a small number of other studies. In the exceptions, subcutaneous injection was used in one study finding a significant response. Oral dosing was used in the other two, with a significant but weak response being found in one gavage administration study and no effect resulting from dietary administration for 6 days. The dietary concentration (0.8% hydroquinone) was the same

as that used for the induction of renal tumours in F344 rats and, with less clarity, hepatocellular adenomas in male B6C3F1 mice (McGregor, 2007).

There are no germ cell studies of hydroquinone mutagenicity in which routes other than i.p. have been used. Unlike the adult human anatomy, the testes of adult rats are likely to receive direct exposure to a chemical that is delivered by the i.p. administration because the inguinal canal remains open. The i.p. route would seem to largely destroy any rationale for testing *in vivo* because exposure of many *in vivo* target cells in animals dosed by this method differs little from that achieved *in vitro*. Although it is not clear that dominant lethal assays are truly germ-cell mutation assays, they are usually assumed to be so. The only one conducted with hydroquinone did not produce any significant response after dosing of male Sprague-Dawley rats with up to 300 mg/kg body weight per day, 5 days per week, for 10 weeks (McGregor, 2007).

Hydroquinone is mutagenic *in vitro* and *in vivo*, but the administration route used to demonstrate the *in vivo* activity is inappropriate and exposure by more appropriate routes, methods and doses allow protective mechanisms to contain the potentially damaging effect of the oxidative properties of hydroquinone (McGregor, 2007).

3.3.4. Carcinogenicity

Bearberry leaf extract

No data available.

Arbutin

No data available.

Hydroquinone

The health effects of hydroquinone have been extensively reviewed in IPCS Environmental Health Criteria No. 157 and summarised in IPCS Health and Safety Guide No. 101 (IPCS, 1994; IPCS, 1996; NTP, 2006).

In a 2-year carcinogenesis bioassay, hydroquinone (25 or 50, or 100 mg/kg) administered by gavage gave some evidence of carcinogenicity in male F344/N rats (kidney tubular cell adenoma), female F344/N rats (mononuclear cell leukaemia), and female B6C3F1 mice (liver adenoma or carcinoma). No evidence of carcinogenetic activity was observed in male B6C3F1 mice after administration of 50 or 100 mg of hydroquinone . Additionally, the incidence of thyroid follicular cell hyperplasia was increased in female and male mice (WHO, 2002; NTP, 2006).

McGregor (2007) refers in his review to U.S. National Toxicological Program Study. In the study, groups of 55 male and 55 female Fischer 344/N rats, 7 to 9 weeks of age, were administered 0, 25, or 50 mg/kg body weight hydroquinone (purity >99%) by gavage on 5 days per week for 103 weeks. Survival was reduced in exposed rats. Also, the mean body weight of exposed males was reduced and the relative kidney weights for high dose males were greater than those for vehicle controls. Nephropathy was observed in nearly all male and most female rats of all dosed groups and vehicle controls. The nephropathy was characterised by degeneration and regeneration of tubule epithelium, atrophy and dilatation of some tubules, hyaline casts in the tubule lamina, glomerulosclerosis, interstitial fibrosis, and chronic inflammation. In males, the nephropathy was more severe in the high-dose (50 mg/kg body weight per day) group, while in females no dose dependence was observed. Nephropathy had also been observed in males in earlier 13-week studies. The presence of hyaline droplets was not reported. In exposed males, renal tubule-cell adenomas developed in 4/55 low-dose

group rats (p=0.069) and 8/55 high-dose group rats (p=0.003), compared with 0/55 in control group rats. A low, non-significant incidence of renal tubule hyperplasia also was reported in the high dose group of male rats (2/55) compared with none in the other groups. There were no renal tumours found in female rats of any group.

A reanalysis of the histology of the NTP study, in addition to demonstrating a low incidence of foci of atypical tubule hyperplasia and small adenomas at both doses, also found substantial exacerbation of chronic progressive nephropathy (CPN) to end stage grades of severity at the high dose (Hard *et al.,* 1997). Briefly, CPN begins at about 2 months of age, when some rats develop basophilic renal tubules with a thickened basement membrane. Progression involves an increase in number of tubules affected, tubule degeneration and atrophy, and an ongoing tubule-cell proliferation in which mitotic figures may be frequent (Hard and Seely 2005, McGregor 2007). By the time that end stage (grade 8) is reached, there are virtually no normal tubules remaining and death from renal failure is highly probable. It is important to recognise that this degenerative and regenerative disease is not the result of any chemical treatment, and it is necessary to distinguish its regenerative aspects from preneoplasia (atypical hyperplasia), from which adenomas develop. These are usually small (≤0.5 mm). In general, the overall low incidence of both atypical hyperplasia and tubule-cell adenomas may be increased by examining additional step-sections of the kidney rather than relying exclusively on the more usual single, cross and longitudinal section.

The histopathological reanalysis of the hydroquinone study (Hard *et al.,* 1997, McGregor 2007) found that of the 8 tumours identified by NTP in the high dose, 4 were definite adenomas (one being a cystadenoma), 3 were very early adenomas (incipient adenomas), and 1 was a focus of atypical hyperplasia. In the low dose, 3 of the 4 tumours diagnosed by NTP were similar to the high dose tumours diagnosed by Hard *et al.*, 1997, 2 being definite adenomas and 1 a very early adenoma; the fourth was considered to be a metastasis from a mesothelioma that was present in the peritoneal cavity and certain lymph nodes in this rat, which died at 56 weeks. Besides the 1 focus of atypical hyperplasia already mentioned, Hard *et al.,* 1997 found 13 other foci of atypical hyperplasia in 11 of the 51 rats examined, whereas only 2 were mentioned in the NTP report. Only one of these was confirmed in the re-evaluation, with the other probably representing an inflammatory lesion unrelated to neoplasia. In the high dose group, 40% of males showed exacerbation of CPN to end stage, compared with 5% in the control group males.

3.3.5. Reproductive and developmental toxicity

Bearberry leaf extract

No data on reproductive and developmental toxicity is available.

Arbutin

Arbutin was administered subcutaneously at 25, 100 or 400 mg/kg of body weight daily to male and female Sprague-Dawley rats before mating and to female rats during pregnancy and lactation. No effect on reproduction of male and female rats, or the development of the offspring was observed at doses of up to 100 mg/kg body weight. However, significant reduction of body weight of female foetuses at Day 20 of pregnancy was observed at doses of 400 mg/kg body weight. Autopsy performed at age 7 and 10 weeks revealed a significant decrease in weight of the left ovary and its ratio to the body weight in females of the 400 mg/kg group.

In the study, the maximum no-effect dose of arbutin was estimated to be 100 mg/kg per day with respect to the reproduction of male and female rats (Itabashi *et al*, 1988).

Hydroquinone

The overall *in vitro* and animal toxicity database indicates that hydroquinone may cause maternal toxicity, which is essentially the same as that seen in non-pregnant animals given similar exposure to acutely toxic dose levels of hydroquinone. Fetotoxicity primarily manifested as growth retardation may be seen at high-dose levels that also induce maternal toxicity. However, reproductive toxicity and/or teratogenic effects are not prominent even at high exposure levels (DeCaprio, 1999).

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

Immunotoxicity

Arbutin

Oral application of arbutin (10 or 50 mg/kg [0.037 or 0.18 mmol/kg]) quickly reduced the swelling caused by picryl chloride and sheep red cell delayed type hypersensitivity in mice within 24 hours (Matsuda *et al.,* 1990, 1991 [Japanese, abstract]). Arbutin (1 mg/ml [4 mM]) inhibited the binding of mouse monoclonal anti-dinitrophenyl immunoglobulin E (IgE[aDNP]) to DNP by 65% (Varga *et al.,* 1991).

In macrophage cells from male Swiss mice, arbutin (2 mg/ml [7 mM]) failed to induce the release of hydrogen peroxide (Moreira *et al.,* 2001; NTP 2006).

Cytotoxicity

Arbutin

Growth of human melanoma cells and normal human melanocytes was not inhibited by exposure to 100 µg/ml [0.367 mM] arbutin for 5 days. At 300 µg/ml [1.10 mM] arbutin treatment for 5 days, cell toxicity and detachment of cells from the dishes were observed within 48 hours (NTP 2006).
Arbutin (5 – 50 µM [1 – 14 µg/ml]) inhibited the growth of the roots of *Allium sativum* L. and produced anti-mitotic effects. At 10 µM, the anti-mitotic effect was already visible within 24 hours and ended at 48 hours. At 20 µM [5.4 µg/ml], arbutin was very active, completely stopping root growth after day 1. The effects were similar to those seen with hydroquinone at 5 µM (Deysson & Truhaut, 1957; NTP, 2006).

Hydroquinone

Hydroquinone has been reported to show a concentration-dependent cytotoxic activity on cultured rat hepatoma cells (HTC line). A dose 33 µg/ml caused cellular mortality of 40% of cells after 24 hours of incubation and no cells remained viable after 72 hours. A higher concentration of 66 µg/ml killed all the cells after a 24-hour contact (Assaf *et al.,* 1987; Barnes *et al.,* 2002).

3.3.8. Conclusions

Bearberry leaf extract

There is almost no information available on the toxicity of crude extract of bearberry leaves.
Bearberry water extract was evaluated in Ames test using only 2 instead of 5 recommended strains. Results of this study were negative; however, clear conclusion about mutagenicity of the extract could not be made.

Bearberry leaf ethanolic extract (extraction solvent ethanol 70%, DER not specified) was also negative in the *in vitro* micronucleus test; however, this test was not performed according to ICH S2B standard. Tests on reproductive toxicity and carcinogenicity have not been performed with bearberry leaves and/or preparation thereof.

Arbutin

Single dose toxicity study has not been performed with arbutin but repeat administration of doses much higher compared to that in clinical practice revealed no toxic effects in mice.

Arbutin has been evaluated *in vitro* in Ames assay and micronucleus test showing no indication of genotoxic potential. The Chinese hamster V79 mutation test performed with arbutin gave negative results. Whilst after preincubation of arbutin with β-glycosidase causing its cleavage to hydroquinone positive results of the assay were found. Furthermore, the *in vivo* mouse micronucleus test revealed no increase incidence of micronuclei formation after treatment with arbutin compared to hydroquinone, used as positive control that gave clear positive results. Arbutin alone seems to be of no genotoxic concern in animal studies, however, combination of arbutin with β -glycosidase but also the observed difference between the whole bearberry leaf extract and pure arbutin should be carefully considered.

Carcinogenicity studies have not been performed with arbutin.

Reproduction and developmental studies performed in rats dosed with arbutin administered subcutaneously showed that there is no risk on male and female fertility and no deaths were observed in offsprings. The results of the study are considered irrelevant to clinical practice since low doses of arbutin were used and a different route of administration was applied.

Hydroquinone

Acute toxicity of hydroquinone has been evaluated and lethal doses were established in several animal species. High exposure of rats to hydroquinone led to severe toxic effect on the central nervous system.

Genotoxicity and mutagenicity of hydroquinone has been extensively studied but clear conclusion could not be made. While the standard Ames assay was negative, sister chromatide exchange, chromosomal aberrations, mouse lymphoma assay and micronucleus test were positive after treatment with hydroquinone. Unambiguous conclusion based on these results could not be made. Firstly, doses of the hydroquinone used in the studies are much higher than is expected as clinical dose after administration of bearberry leaf extract. Secondly, in the *in vivo* studies hydroquinone was mainly administered intraperitoneally. In case of oral administration, results were often negative and toxic effects described as mild. Furthermore, hydroquinone is a naturally occurring substance and the human body is commonly exposed to this substance. Hydroquinone is present in coffee, tea or pears and low parts-per-million levels are detectable in the human body. Finally, after administration of bearberry leaf extract or arbutin, hydroquinone has been detected in human urine only in several studies and in very small amount. After administration of bearberry leaf extract, free hydroquinone in amounts above the detection limit (1 µg /ml – HPLC method) was found only at pH 8. It should also be considered that administration of bearberry leaf to humans is limited to very short period and the time of exposure of human body to hydroquinone is also very short since it is rapidly transformed to its inactive and nontoxic metabolites that are excreted via urine.

Free hydroquinone showed no mutagenetic risk in several *in vitro* and *in vivo* assays. The potential mutagenicity of free hydroquinone occurred only at concentrations that excess more than 20 times the maximal theoretical concentration reached in an animal or human organism (2.4 mg/kg; when all arbutin was transformed in free hydroquinone). As a response to this possibility, the organism has a potent conjugation metabolism to neutralise the hydroquinone just after its formation.

The safety margin could be considered sufficient regarding toxicity and adverse effects.

Furthermore, it should be considered that available data did not report any serious adverse and toxicity effects in animals after administration of arbutin or hydroquinone doses relevant for human use and assessment of human safety.

No adverse toxic reactions at the doses comparable with the recommended dose of bearberry leaves have been reported in scientific literature and several types of bearberry leaf extract have been in medicinal use for many years. Therefore, non-clinical data on bearberry leaf extract and its main components arbutin and hydroquinone can be considered sufficient to support the traditional use of bearberry leaf extract in the short-term treatment of mild lower urinary tract infections.

3.4. Overall conclusions on non-clinical data

Non-clinical data on antimicrobial activity could be considered supportive for the plausibility of the traditional use.

The ability of bearberry leaf to act against urinary infections is probably the result of action of free hydroquinone cleaved from the arbutin molecule in the urinary tract. The cleavage is mediated via β-glycosidase as described in several *in vitro* tests.

Adequate tests on genotoxicity are not available

Carcinogenicity studies performed with extract of the Uvae ursi leaves and/or arbutin are not available. Toxicity tests with hydroquinone, a hydrolysis product of arbutin, have demonstrated some evidence of genotoxicity and carcinogenicity. However, risks posed by the exposure of hydroquinone during the short-term treatment with Uvae ursi folium preparations are considered minimal.

Reproductive toxicity with bearberry leaf or preparations thereof has not been studied. Nevertheless, arbutin, the principal component of Uvae ursi folium, displayed changes in body weight of female foetuses and in ovary weight in females at age 7 and 10 weeks after subcutaneous administration of the dose 400 mg/kg per day to rats before mating and during pregnancy and lactation. No effect on reproduction has been observed at doses of 100 mg/kg per day. No risk on male and female fertility and no deaths were observed in offsprings. However, the results of the study are considered irrelevant to clinical practice since low doses of arbutin were used and different route of administration was applied.

Oral administration of bearberry leaves can be regarded as safe at the recommeded doses.

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

The antiseptic and diuretic properties claimed for bearberry leaf extract can be attributed to the hydroquinone derivatives, especially arbutin. Arbutin is absorbed from the GI tract virtually unchanged and during renal excretion is hydrolysed to yield the active principle, hydroquinone, which exerts antiseptic and astringent action on the urinary mucous membranes (Frohne, 1970).

Arbutin

In a study without controls, urine samples from healthy volunteers were collected 3 hours after oral administration of 0.1 or 1 g arbutin. The urine samples (adjusted to pH 8) and 20 antibacterial compounds (at their usual urine concentration) were tested *in vitro* using 74 strains of bacteria, including *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Only arbutin (present in urine samples collected after administration of 1 g arbutin), gentamicin and nalidixic acid were active against all the strains tested. An antibacterial effect was observed also at 10-fold lower doses of arbutin (0.1 g). The maximum effect was detected from 3 – 4 hours post-dose. The effect of arbutin was higher in alkaline urine in comparison to urine with pH 6. It could be concluded that metabolic products of arbutin are responsible for antibacterial effect of bearberry leaves but these substances are active only in alkaline urine (Kedzia *et al.,* 1975; WHO, 2002).

Oral administration of arbutin (800 mg) or an infusion of the leaves containing equivalent amount of arbutin to healthy volunteers had strong antibacterial activity against *Staphylococcus aureus* SG 511 and *E. coli*, as measured in urine samples after adjustment of the urine pH to 8.0. Urine of pH 6 was ineffective (Frohne, 1970). With respect to the slight toxicity of arbutin, its metabolic product could be used as an effective antibacterial substance in the alkaline environment against bacterial infection of the urinary tract (Frohne, 1977).

The antibacterial effect of bearberry leaf extract according to the hypothesis formulated already in 1883 is ascribed to hydroquinone which is liberated from arbutin via glycoside cleavage. Therefore, arbutin is the prodrug of the relatively toxic active principle hydroquinone. In the case of arbutin, hydrolysed by β-glucosidase, the formed aglycone hydroquinone may possibly be absorbed already in the intestine or in the liver and detoxified through conjugation with glucuronic acid or sulfuric acid, these conjugates do not have antibacterial activity, but they can be hydrolysed by bacterial enzymes (Frohne, 2004).

Hydroquinone

In urine hydroquinone exists in a form of glucuronide and after alkalinisation of the urine, it splits to the free form having antibacterial activity (ESCOP, 2003).

It is still unknown which of the hydroquinone compounds are responsible for the antibacterial effect. Investigation data revealed that hydroquinone glucuronide could not be responsible for antibacterial activity since its maximum in urine was detected 2 hours after administration of arbutin and maximum antibacterial activity of urine was observed at 3 to 4 hours post-dose. This led to the conclusion that the most probable compound responsible for the antimicrobial activity could be hydroquinone sulphate instead of hydroquinone glucuronide (Kedzia *et al.,* 1975).

Pharmacodynamic drug interactions

Bearberry leaf extract

There were no drug interactions documented for bearberry leaf extract. However, it was observed that the sodium sparing effect of bearberry leaf extract may offset the diuretic effect of thiazide and loop diuretics (Gruenwald *et al.,* 2004). As information on sodium sparing effect is not supported by any published case report, this interaction is not included in the European Union herbal monograph.

Alkalisation of urine

Urine samples from healthy volunteers received arbutin-containing tea or pure arbutin showed after alkalinisation of the urine inhibition of growth of bacteria tested. Samples of normal urine with or without alkalisation and arbutin solutions (1 – 3 mg/ml) did not show any antibacterial activity (Frohne

1970). It has been experimentally proven that pH value of the urine sample is very important for its antibacterial activity. Antibacterial effect of arbutin was increased and prolonged in alkaline urine pH 8 (when compared to the urine sample at pH 6) (Kedzia *et al.,* 1975).

Urine with high content of metabolic products of arbutin leaving on the air clearly showed low potency for bacterial infections compared to the control urine sample. Bacterial culture incubation (*E. coli* and *Staphylococcus aureus*) showed a clear inhibitory effect of urine containing metabolic products of arbutin. This urine sample has to be alkaline. Alkalisation alone or addition of arbutin alone did not show any bacteriostatic effect in the sample of urine (Frohne, 1977).

Whether the alkalising of the urine – which, through the administration of sodium hydrogen carbonate, can be attained only short-term – also has the same effect *in vivo*, is doubtful. In any case, measurable levels of hydroquinone have not been detected in the urine (Paper *et al.,* 1993; Frohne 2004).

A problem is the way of making the urine environment more alkaline. The common daily dose of natrium hydrogencarbonate or dinatrium phosphate is able to alkalinise urine only for a short period of time. Kedzia *et al.*, 1975 suggested using acetazolamide which is able to make urine alkaline for longer period; however, due to its toxicity, it could be administered only for 3 or 4 days. Frohne stated that further investigation of how to alkalinise urine in a much more effective manner is necessary (Frohne, 1977).

Since concomitant acidification of the urine (by other remedies) may result in a reduction of its antibacterial efficacy, several references included statements on patients being advised to avoid eating highly acidic foods, such as acidic fruits and their juices during treatment with Uvae ursi folium (WHO, 2002; Barnes *et al.,* 2002; ESCOP 2003; Gruenwald *et al.,* 2004).

However, a study from 2003 demonstrated that bacteria causing urinary tract infections participate in the deconjugation of arbutin and liberate the toxic free hydroquinone. The free hydroquinone then can damage the cell by destabilisation of its membranes. Alkalisation of the urine by intake of sodium bicarbonate is not necessary considering the effective bacterial deconjugation by *E. coli*, the principal agent in urinary infections (Siegers *et al.,* 2003).

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

The main constituent of Uvae ursi folium extracts is arbutin, a phenolic glycoside, splits in hydroquinone and glucose (Jahodář *et al.,* 1985). Pharmacokinetic studies have been mainly focused on the availability of total hydroquinone in the urine. The use of hydroquinone and arbutin as pharmacokinetic markers is reasonable and justified (Paper *et al.,* 1993; Schindler *et al.*, 2002; Quintus *et al.,* 2005).

Bearberry leaf extract

After ingestion of the leaves, arbutin is absorbed from the GI tract, and is hydrolysed by intestinal flora to form the aglycone, hydroquinone (Paper *et al.,* 1993). Hydroquinone is metabolised to glucuronide and sulphate esters that are excreted in the urine (Kedzia *et al.,* 1975; Frohne 1970). These active hydroquinone derivatives exert an antiseptic and astringent effect on the urinary mucous membranes when the urine is alkaline (pH 8). Their antibacterial action reaches a maximum approximately 3 – 4 hours after ingestion (Blumenthal *et al.,* 1998; Paper *et al.,* 1993).

In a study with one healthy volunteer, four hours after ingestion of a single dose of a preparation containing bearberry leaf extract (945 mg corresponding to 210 mg arbutin), 224.5 µmol/L

hydroquinone glucuronide and 182 µmol/L hydroquinone sulphate were recovered in the urine, which represented approximately half of the administered arbutin dose (Glöckl *et al.,* 2001).

The bioavailability of gastro-resistant coated tablets containing aqueous extract of Uvae ursi folium in comparison to the genuine extract was studied in a crossover design with 6 healthy volunteers. The arbutin equivalent was determined in urine samples collected within 24 hours, using the DAB 10 spectrophotometric method. The release of arbutin from the tablet compared to the extract was retarded by at least 3 hours. However, the bioavailability showed comparable values. In the urine samples, no free hydroquinone was detected using HPLC analysis. In a pilot study, the coated tablets and extract were administered together with 10 g sodium hydrogen carbonate. The pH of the urine changed from 6.5 to 7.4 and in one case to pH 8 for one hour. Free hydroquinone was found in a therapeutic concentration in urine only if the pH was alkaline (pH 8). In other urine samples, the hydroquinone concentrations were below detection limit (1 µg/ml – HPLC method) (Paper *et al.,* 1993). These findings are in agreement to those by Frohne, who detected free hydroquinone only after adjusting samples to pH 8 (Frohne, 1970).

In an open, randomised, two-way crossover study, 16 healthy volunteers (8 males, 8 females, mean age of 25.4-year old) received a single oral dose of bearberry leaf dry extract (BLDE) as film-coated tablets (2 tablets containing 472.5 mg BLDE, corresponding to 105 mg arbutin) or as an aqueous solution (945 mg BLDE, corresponding to 210 mg arbutin). Hydroquinone glucuronide and hydroquinone sulphate were recovered in the urine during the first 4 hours but no metabolites were detected after 24 hours. The rate of metabolism was faster in the group given BLDE in an aqueous solution. The total metabolite concentration represented 66.7% of the administered dose in the tablets and 64.8% in the solution. Hydroquinone glucuronide accounted for 67.3% and 70.3% of the total arbutin metabolites recovered in each group, respectively (Schindler *et al.,* 2002).

Twelve human volunteers (6 males and 6 females) received 3 x 2 coated tablets containing 238.7 – 297.5 mg of bearberry leaf dry extract (DER 3.5 – 5.5:1, extraction solvent ethanol 60% V/V corresponding to 70 mg of arbutin in one tablet). The urine was sampled for 36 hours and fractionated in periods of 6 hours. Free and conjugated hydroquinone was measured in the samples. Only 0.6% of the administrated arbutin dose (420 mg) was excreted as free hydroquinone and in 6 out of 12 volunteers no free hydroquinone was detected in urine (detection limit 0.3 µg/ml); 70% of the arbutin dose was found as hydroquinone conjugated to glucuronic and sulfuric acid. Urine samples collected in this study were assayed with or without added glusulase (mixture of β-glucuronidase, aryl-sulfatase and cellulase) or an *E. coli* suspension, and analysed by HPLC for hydroquinone content. Incubation of the urine with *E. coli* proved the ability of bacteria to deconjugate the hydroquinone glucuronide and sulphate to free hydroquinone. Deconjugation was 2.3-fold higher than after incubation with glusulase (Siegers *et al.,* 1997, 2003).

Arbutin

As arbutin is reported to hydrolyse easily in diluted acids to yield D-glucose and hydroquinone, it is expected that ingested arbutin would be hydrolysed to free hydroquinone by stomach acids. However, a set of experiments suggested that absorbed hydroquinone is rapidly conjugated since it is not detectable as free hydroquinone. The level of free hydroquinone in the body (urine and plasma samples) is usually at or below total concentration of hydroquinone measured in the pre-exposure background samples (Deisinger *et al.,* 1996).

Arbutin was found to be extensively absorbed from the GI (gastrointestinal) tract and bioavailable as hydroquinone. Volunteers (2 males and 2 females, 36 – 45 years old) receiving a diet containing high levels of arbutin and hydroquinone (coffee or tea, wheat cereal, whole wheat bread, wheat germ and Bosc pears) had significant increases in mean total hydroquinone (i.e., hydroquinone and its

conjugated metabolites) plasma levels. After 2 hours, hydroquinone was 5 times the background concentration (at 0.15 µg/g [0.55 nmol/g]). Urinary total hydroquinone excretion rates were also significantly increased; after 2 to 3 hours, levels were 12 times background levels. A low-hydroquinone diet (corn cereal, 2% milk, cantaloupe, black cherry yogurt and soft drink) resulted in a slight decrease in the mean levels of hydroquinone in human plasma and urine (Deisinger *et al.,* 1996).

Hydroquinone

Most studies of hydroquinone kinetics and metabolism following oral administration have used arbutin or another form of bearberry leaf extract. For information on hydroquinone kinetics, see sections above.

4.2. Clinical efficacy

4.2.1. Dose response studies

Bearberry leaf extract

No data available

Arbutin

Dose response has been investigated in healthy volunteers. Antibacterial effect has been observed after administration of 0.1 or 1 g of arbutin. Lower dose provided lower antibacterial effect but, independently of the dose, the pH value of urine was the most important factor determining the antibacterial activity (Kedzia *et al.,* 1975).

According to Frohne (1970), the effective concentration of arbutin should be higher than 0.3%.

4.2.2. Clinical studies (case studies and clinical trials)

Clinical research assessing the effects of bearberry leaf extract as a single substance/preparation is not available at the time of the preparation of this report.

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

There are no clinical studies in special population available for bearberry leaf extract or for arbutin.

4.4. Overall conclusions on clinical pharmacology and efficacy

There are no clinical studies evaluating the efficacy of bearberry leaf extract that would be suitable to support the well-established use of this extract. Nevertheless, based on the results of *in vitro* tests on antimicrobial activity of bearberry leaf preparations containing arbutin as a main component and long-standing use of bearberry leaf and preparations thereof for treatment of uncomplicated infections of the lower urinary tract, the traditional use of bearberry leaf extract in the short-term treatment of uncomplicated lower urinary tract infections can be considered plausible.

5. Clinical Safety/Pharmacovigilance

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

There is a lack of clinical safety and toxicity data for bearberry leaf extract and further investigation of these aspects should be performed.

5.2. Patient exposure

Aside from market presence and data from studies there is no special data on patient exposure to bearberry leaf extract available.

No special data is available on patient exposure to arbutin.

Several cohort studies were performed with workers getting in daily contact with hydroquinone. Workers were engaged in colour printing and processing, in a plant where hydroquinone was manufactured, as lithographers or in motion picture film processing. The most serious human health effect related to hydroquinone is pigmentation of the eye and, in a small number of cases, permanent corneal damage. This effect has been observed in HQ (hydroquinone) production workers, but the relative contributions of HQ and BQ (*p*-benzoquinone) to this process have not been delineated. Corneal pigmentation and damage has not been reported at current exposure levels of <2 mg/m^3. Epidemiological studies with hydroquinone have demonstrated lower death rates and reduced cancer rates in production workers when compared with both general and employed referent populations. Hydroquinone is also used in cosmetics and prescription depigmenting skin creams (DeCaprio, 1999). Hydroquinone is also present at significant levels in cigarette smoke and in certain fruits and vegetables (in the form of its glucose conjugate, arbutin), including pears and blueberries and in coffee beans and certain wheat-based products. Low levels of hydroquinone were detected even in urine of unexposed persons which can be attributed to hydroquinone containing foods or, alternatively, to environmental exposure to benzene or phenol (Deisinger *et al.,* 1996, DeCaprio, 1999, Garcia de Arriba *et al.,* 2013, NTP 2009, McGregor 2007).

Dietary sources of hydroquinone/arbutin as well as other products such as cigarettes consumed daily by many humans have shown to generate comparable or even higher exposure levels to free hydroquinone than to the recommended dosage of Uvae ursi folium and preparations thereof. It should be noted that these products are consumed many times a day over the course of a lifetime, by all segments of the overall population. The level of free hydroquinone produced by the administration of the recommended dose of Uvae ursi folium or preparations thereof was estimated to be in the order of 11 mg/kg bw/d. This exposure level represents 11% of the permitted daily exposure (PDE) below which there is a negligible risk to human health (Garcia de Arriba *et al.,* 2013).

5.3. Adverse events, serious adverse events and deaths

The oral administration of preparations of Uvae ursi folium may cause nausea and vomiting due to stomach irritation from the high tannin content (WHO, 2002; Blumenthal *et al.,* 1998; Gruenwald *et al.,* 2004; British Herbal Pharmacopoeia 1996; ESCOP 2003; Bradley 1992). Stomach ache has also been reported as adverse effect (Gruenwald *et al.,* 2004; Hänsel *et al.,* 1993).
In view of the high tannin content and toxicity of hydroquinone, prolonged use of bearberry leaf extract may cause chronic liver impairment (Barnes *et al.,* 2002; Gruenwald *et al.,* 2004).

Due to the high tannin content of the leaves, in persons with sensitive stomach, nausea and vomiting are possible side effects after ingestion of dried bearberry leaves (as low as 15 g) or tea infusion (Frohne, 2004). Other symptoms that have been reported in association with bearberry leaf extract are irritability, insomnia and increased heart rate (NTP, 2006).

Bearberry leaf extract use has also been linked to albuminuria, haematuria and urinary cast (Adesunloye, 2003; NTP, 2006).

In a 56-year old female who drank tea containing bearberry leaf regularly for 3 years to prevent recurrent urinary tract infection, bull's eye maculopathy, paracentral scotomas, reduction in electroretinography amplitude and retinal thinning on optical coherence tomography were observed.

The maculopathy was suspected to result from bearberry leaf because of its ability to inhibit melanin synthesis (NTP 2006).

Uvae ursi folium should not be used for prolonged periods. Patients with persistent symptoms of a urinary tract infection should consult a physician. Use of Uvae ursi folium may cause a greenish-brown coloration of the urine that darkens on exposure to air due to the oxidation of hydroquinone (WHO, 2002).

5.4. Laboratory findings

Laboratory screening tests were performed within an open, randomised, two-way crossover study with 16 healthy volunteers who received a single oral dose of bearberry leaf dry extract as film-coated tablets (2 tablets containing 472.5 mg dry extract, corresponding to 105 mg arbutin) or as an aqueous solution (945 mg dry extract, corresponding to 210 mg arbutin). The tests were performed before the treatment and included a differential blood count, prothrombin time (PT), partial thromboplastin time (PTT), electrolytes (potassium, sodium, calcium, magnesium), blood glucose, urea, creatinine, bilirubin, aspartate-amino-transferase, alanin-amino-transferase, gamma- glutamyl-transferase, alkaline phosphatase, alpha-amylase, total protein, albumin, C reactive protein, triglycerides, cholesterol, and semi quantitative urinalysis. After the treatment period, the laboratory parameters were screened again, except for PT, PTT, triglycerides and cholesterol. There were no unexplained pathologic laboratory results that were suspected to be the result of the treatment with bearberry leaf dry extract (Schindler *et al.,* 2002).

5.5. Safety in special populations and situations

The safety of bearberry leaf extract in human is mainly based on the traditional use. There are only several clinically reliable data regarding safety of the extract after administration in human.

Incidence of urinary tract infections (UTIs) in men

UTIs are the second most common form of infection, accounting for nearly 25% of all infections. Incidence of UTIs is significantly higher in women than in men. Incidence of UTIs in men is increasing with age. Asymptomatic bacteriuria is believed to affect up to 50% of geriatric women and 30% of geriatric men. Asymptomatic bacteriuria is prevalent in elderly population but it frequently resolved without treatment and has no long-term sequelae. However, symptomatic UTIs among the elderly requires antimicrobial therapy (Foxman, 2002).

For males aged 17 – 79 years, the mean annual UTIs incidence is 2.2%, for males aged ≥ 80 years, the mean annual UTIs incidence rises to 5.3% and risk factors in this age include presence of an indwelling urinary catheter, hospitalization and anatomical abnormalities associated with aging or disease (e.g. benign or malignant prostatic hyperplasia). In women aged 15 – 39 years, the mean annual UTI incidence is 15.2% and with age falls to 11.4% for women aged 40 – 59 years and to 9.7% for women aged 60 – 79 years (Guay, 2008). Guay (2008) also conducted epidemiological study of UTIs in a non-selected population-at-large. In this study, laboratory surveillance was conducted for all community-acquired UTIs among residents of Calgary Health Region (population of 1.2 million) in Canada during 2004 and 2005. A total of 40,618 episodes of UTI occurred among 30,851 residents (overall annual incidence was 17.5/1000). The incidence was significantly higher in females than in males (30.0 *vs.* 5.0/1000). After the first year of life, UTI was rare in males until late middle age, at which point a significant increase occurred with each subsequent decade of life. In the very old age (80 – 89 years old), UTI was common (males 638, females 926, overall 851/1000/year) (Guay, 2008).

Much higher incidence of UTIs in females during adolescence and childbearing years (adult women are 30 times more likely than men to develop a UTI) is reported also by Brusch (2011). The frequency of UTI in men approaches that of women only in men older than 60 years; in men aged 65 years or older, 10% have been found to have bacteriuria, as compared with 20% of women in this age group. In the normal host, UTI in men may occur due to infection of other parts of the genitourinary tract, typically the prostate. Older males with prostatic hypertrophy have incomplete bladder emptying, predisposing them to UTI on the basis of urinary stasis. However, in males aged 3 months to 50 years, the incidence of UTI is low; therefore, the possibility of anatomic abnormality must be considered. In males older than 50 years, prostatic hypertrophy with partial obstruction is the main contributor to the increase UTI (Brusch, 2011).

Among complications of benign prostatic hyperplasia (BPH) resulting from persistent failure of the bladder to empty or store urine recurrent urinary tract infections are mentioned (Lee, 2000). In patients with mild symptoms of BPH, whose symptoms do not cause unacceptable distress, no drug therapy with α-adrenergic antagonists or with finasteride is yet necessary, and watch-full waiting is indicated instead (Lee, 2000; Clifford and Farmer, 2000). For treatment of symptoms of mild recurrent lower UTIs short term use of bearberry products can be recommended but only after consultation with a doctor.

Neisseria gonorrhoeae and *Chlamydia trachomatis* are clinically important infectious causes of urethritis (Workowski and Berman, 2006).

Assessor´s conclusion:

According to reports on the traditional use no differentiation between the genders was done. Recurrent mild infections of the lower urinary tract are usually uncomplicated in women. In men, due to the anatomical disposition of the lower urinary tract, a risk of severe inflammations of lower urinary tract of various origin exists and therefore urinary tract infection always requires medical examination.

A delayed consultation of a medical doctor may imply serious risks for men. In men over 50 years, incidence of UTIs is increasing due to prostatic hyperplasia (benign or malignant) and presence of an indwelling catheter. Both, prostatic hyperplasia and indwelling catheter require medical supervision.

The criterion of the Article 16 a) of Directive 2001/83/EC for traditional herbal medicinal products "they have indications exclusively appropriate to traditional herbal medicinal products which, by virtue of their composition and purpose, are intended and designed for use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment" is not fulfilled for use in men. Therefore, the use in men is excluded from the traditional use and traditional use can be recommended for females only.

Although men are excluded from the traditional use, Uvae ursi folium or preparations thereof can be used when advised by a medical doctor.

5.5.1. Use in children and adolescents

A posology for children and adolescents was published in "Kinderdosierungen von Phytopharmaka" (Dorsch *et al.,* 1998) for bearberry leaf:

Dosage in children:

0 – 1 year	>1 – 4 years	>4 – 10 years	>10 – 16 years
		5 – 10 g	10 g

Corresponding amount of arbutin:

0 – 1 year	>1 – 4 years	>4 – 10 years	>10 – 16 years
			400 – 700 mg

However, posology data for children is not supported by any clinical data (clinical studies, post marketing reports). The data published by Dorsch *et al.,* 1998 are derived from calculations only.

Furthermore, the use in children and adolescents cannot be recommended for traditional use because infections of the urinary tract, even in their early stage, in children and adolescents should be treated under medical supervision.

5.5.2. Contraindications

Bearberry leaf and the preparations thereof should not be used by patients with known hypersensitivity to the herbal substance.

Following phytotherapeutic monographs such as ESCOP (2003) and WHO monograph (2002), monograph *Arctostaphylos uva-ursi*/Uva ursi in British Herbal Pharmacopoeia (1996), PDR for Herbal medicines (Gruenwald *et al.,* 2004) and British Herbal Compendium (Bradley, 1992) the use in case of kidney disorders is contraindicated.

Bearberry is listed among the supplements and herbal remedies which should not be used in chronic kidney disease (Management of Chronic Kidney Disease, 2015).

5.5.3. Special warnings and precautions for use

The use in children and adolescents under 18 years of age is not recommended because of concerns requiring medical advice.

The use in men is not recommended because of concerns requiring medical supervision.

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.

Uvae ursi folium may cause a greenish-brown coloration of the urine.

5.5.4. Drug interactions and other forms of interaction

There are no drug interactions documented for bearberry leaf or preparations thereof.

Inhibitory effect of aqueous and methanolic extracts on CYPA3 isoenzymes was proved by Chauhan *et al.,* 2007 in an *in vitro* test (for details see section 3.2). Nevertheless, as information on effect on CYPA3 isoenzymes is not supported by any published case report, this interaction is not included in the European Union monograph.

5.5.5. Fertility, pregnancy and lactation

No fertility data is available.

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

5.5.6. Overdose

An overdosing with bearberry leaf extract can lead to inflammatory irritation of the bladder and urinary tract mucosa accompanied with dysuria and haematuria, or later with blood excreta. Finally,

overdosing could lead to liver damage (hepatic impairment) (Gruenwald *et al.,* 2004; Hänsel *et al.,* 1993).

Extremely high doses of bearberry leaf extract (i.e., ten times the recommended amount) can cause tinnitus, shortness of breath, convulsions, collapse, delirium, and vomiting. Liver damage is a risk with long-term use (NTP, 2006).

Large doses of bearberry leaf extract are reported to be oxytocic, although *in vitro* studies have reported a lack of utero-activity (Barnes *et al.,* 2002). Although the symptoms of overdose and long-term use are described in the literature, they are not supported by case reports. Taking into consideration information from the literature, the patients are advised to follow the recommended dose and duration of use.

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

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

5.5.8. Safety in other special situations

For use in men see section 5.5.

5.6. Overall conclusions on clinical safety

There are no clinical safety data available for bearberry leaf and preparations thereof. Nevertheless, in some of literature sources the adverse reactions (gastrointestinal complaints) are attributed to tannins which are presented in bearberry leaves in relatively high amount (10 to 20 %). To reduce elution of tannins as much as possible it is recommended to prepare herbal tea from bearberry leaves by maceration or infusion and not by decoction. The macerate should be used immediately after preparation.

Based on long experience with herbal medicinal product used with the daily dose of the extract corresponding to 840 mg of hydroquinone derivatives, use bearberry leaf and preparations thereof in the doses corresponding to 840 mg of hydroquinone derivatives calculated as arbutin per day for one week can be considered as safe for human use.

6. Overall conclusions (benefit-risk assessment)

Based on the data documented in the assessment report, a European Union herbal monograph is established on the traditional uses of several preparations of *Arctostaphylos uva-ursi* (L.) Spreng., folium. Scientific evidence of its efficacy and safety in human is very poor and considered insufficient to support well established use of the herbal substance and extracts. The traditional uses of Uvae ursi folium preparations fulfil the requirement for at least 30 years of medicinal use at a specified strength and specified posology, according to Directive 2001/83/EC. Nevertheless, the criterion of the Article 16 a) of Directive 2001/83/EC for traditional herbal medicinal products “they have indications exclusively appropriate to traditional herbal medicinal products which, by virtue of their composition and purpose, are intended and designed for use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment” is not fulfilled for use in men, as the use in men requires medical supervision. Therefore, the use in men is excluded from the traditional use and traditional use can be recommended for women only.

The efficacy is plausible on the basis of long-standing use and experience for the following indication:

Traditional herbal medicinal product used for relief of symptoms of mild recurrent lower urinary tract infections such as burning sensation during urination and/or frequent urination in women, after serious conditions have been excluded by a medical doctor.

Non-clinical data available from the published literature could be considered sufficient to support the above mentioned traditional indication.

Clinical data describing efficacy and safety of bearberry leaf and any preparations thereof or its main component arbutin are not available.

The benefit/risk ratio can be considered positive. The long-standing use of bearberry leaf extracts in the treatment of uncomplicated bacterial infection of the lower urinary tract, the absence of reported serious adverse effects directly attributable to bearberry leaf extracts, and the results of non-clinical *in vitro* and *in vivo* experiments proving antimicrobial activity support the use as traditional herbal preparations under the specified conditions of use and at the recommended dosages.

The only risk apparent from the available literature is the toxicity of hydroquinone. This substance when used in large amount has been reported to be toxic to animals and also mutagenic in some *in vitro* and *in vivo* tests. In any of the studies performed with arbutin and/or bearberry leaf and preparations thereof, hydroquinone was not detected in the samples in the level above 1 mg/ml. This amount is very close to the most conservative Threshold of toxicological concern (TTC) value. Moreover, the time of exposure of human body to hydroquinone is also very limited, since it is rapidly transformed to its nontoxic metabolites that are excreted via the urine. Therefore, the recommended dose of bearberry leaf or preparations thereof is considered safe in short-term use.

No data on fertility are available. Safety during pregnancy and lactation has not been established. In absence of sufficient data, the use during pregnancy and lactation is not recommended.
The use in children and adolescents is not recommended as medical advice should be sought in this age group.

The therapeutic areas for browse search on the EMA website are “Urinary tract and gynaecology disorders”.

Based on the available information arbutin is considered an analytical marker by the HMPC.

A European Union list entry is not supported due to lack of adequate data on genotoxicity.

Annex

List of references