05 May 2021
EMA/HMPC/517879/2016
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Vaccinium macrocarpon* Aiton, fructus

Draft

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Vaccinii macrocarpi fructus
Herbal preparation(s)	Expressed juice from the fresh fruit (DER 1:0.6-0.9)
Pharmaceutical form(s)	Herbal preparations in liquid dosage forms for oral use
Rapporteur(s)	Z. Biróné Dr Sándor
Assessor(s)	O. Roza, E. Widy-Tyszkiewicz
Peer-reviewer	B. Kroes

This draft assessment report is published to support the public consultation of the draft European Union herbal monograph public statement on *Vaccinium macrocarpon* Aiton, fructus. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

1. Introduction

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

Herbal substance(s)

Vaccinium macrocarpon Aiton, fructus – Ericaceae

Monographs in the European Pharmacopoeia or any national pharmacopoeia are not available in the European Union.

There is a monograph 'Cranberry fruit' available in the American Herbal Pharmacopoeia (Upton, 2002, Upton & Brendler, 2016). According to the text, Cranberry fruit (fructus macrocarponii) consists of the fresh or dried whole, crushed, or powdered mature fruits of Vaccinium macrocarpon Aiton conforming to the methods of identification and standards provided. WHO monograph (2009) and ESCOP monograph (2009) also give the definition of the herbal substance referring to American Herbal Pharmacopeia (2002).

Vaccinium macrocarpon is a trailing, often ascending, evergreen shrub to 5–20 cm tall. The fruit is 4-loculed, globose, 9–20 mm in diameter; glabrous; red to crimson, dark burgundy, or almost black; several- to many-seeded. It is native to eastern North America from Newfoundland south to North Carolina and west to central Minnesota. Cultivated and/or escaped in other parts of North America and in Britain and Europe, especially Germany, Switzerland, parts of Eastern Europe, and the Netherlands, Chile, China, and New Zealand (Upton & Brendler, 2016).

Constituents

Cranberries (*Vaccinium macrocarpon, V. oxycocus and V. eruthrocarpum)* are composed of 88% water, yet the active subfraction of the low-polarity concentrate fraction has at least 248 individual constituents (Guay, 2009). Organic acids: At least 14 organic acids are represented in cranberries, e.g. quinic malic or citric acid (Guay, 2009).

Vitamins: High level of Vitamin C (200 mg/kg fresh berries) (Guay, 2009)

Iridoid glycosides are responsible for the taste of cranberry products, e.g. monotropein, coumaroyl (Guay, 2009).

Phenolic acids. Cranberry also contains phenolic acids, including hydroxybenzoic and hydroxycinnamic acids (Blumberg *et al.,* 2013).

Triterpenoids Ursolic acid and derivates (Blumberg *et al.,* 2013)

Anthocyanins are the red pigments found in cranberries. The anthocyanins found in cranberries are the arabinose, galactose and glucose 3-*O*-glycosides of the 6 aglycones: cyanidin, peonidin, malvidin, pelargonidin, delphinidin, and petunidin (Cunningham 2008; Blumberg *et al.*, 2013). The anthocyanin galactosides are most predominant, followed by the anthocyanin arabinosides and very low levels of anthocyanin glucosides. The total anthocyanin content of fresh fruit is ranging from 13.6 to 171 mg/100 g fresh fruit (Upton & Brendler, 2016; Blumberg *et al.*, 2013).

Total anthocyanin content has been shown to vary by growing region with supplies grown in Oregon (70 mg/100 g), Washington (55 mg/100 g), and British Columbia (45 mg/100 g) yielding the highest concentrations, followed by Massachusetts (~42 mg/100 g), New Jersey (~37 mg/100 g), and Wisconsin (~35 mg/100 g) (Upton & Brendler, 2016).

Proanthocyanidins (PACs) or condensed tannins are oligomers and polymers of different flavan-3-ol oligomers with primarily epicatechin units (catechin and (epi)gallocatechins are present only in trace amounts), predominantly in the form of tetramers (49%) and pentamers (37%). Although they can be 4–10 epicatechin units in length, most are four or five such units in length. There are two common series of procyanidin dimers. The "B-type" series are dimers linked either in the C4–C6 or C4–C8 position whereas the "A-type" series are dimers linked in the C4–C8 position with an additional C-2-O-C7 linkage. PACs with at least 1 A-type linkage account for 51–91% of total PACs in cranberry. Cranberries at 100 g fresh weight provide 419 +/- 75 mg total flavan-3-ols, including 70 mg oligomers with DP of 4–6, 63 mg oligomers with DPs of 7–10, and 234 mg polymers, whereas monomers, dimers, and trimers are present at lower amounts (Guay, 2009; Krueger *et al.* 2013a; Blumberg *et al.,* 2013)

Flavonols. Mainly quercetin and myricetin, and to a lesser extent, kaempferol and their 3-*O*-glycosides (Puski & Francis, 1967, Blumberg *et al.,* 2013).

Sugars and Complex Carbohydrates

Total sugar content is 3.6-5.0 g/100g. Cranberries have a high glucose:fructose ratio, which is unusual for fruit juices. Hong and Wrolstad (1986) reported an average glucose:fructose 79%-21%. The detection of sorbitol in significant amounts can also be an indication of adulteration, as sorbitol is only found in cranberry in trace amounts (Hong and Wrolstad 1986).

The cranberry cell wall consists of cellulose, pectin and hemicellulose (Holmes and Rha 1978).

The soluble fiber fraction of cranberries contains oligomeric saccharides.

Soluble oligosaccharides are present at relatively high concentrations (~20% w/w or greater) in many cranberry materials. Cranberry oligosaccharides are mixtures, including xyloglucans and arabinoxyloglucans, pectic acid oligomers, and, possibly, arabinans and arabinoxylans (Marlett and Vollendorf 1994, Coleman and Ferreira 2020). Auker *et al*., 2019 gave a structural characterization of these cranberry arabinoxyloglucan oligosaccharides.

- Herbal preparation(s)

Cranberry juice

A monograph of **cranberry liquid preparation** is published in the United States Pharmacopeia (USP24 2000 and USP 32 2009): bright red **juice** derived from the fruits of *Vaccinium macrocarpon* or *V. oxycoccos* (Ericaceae). It contains no added substances. The pH is between 2.4 and 2.6. The content requirements of the United States Pharmacopoeia include: not less than 2.4% of dextrose, 0.7% of fructose, 0.9% of quinic acid, 0.9% of citric acid and 0.7% of malic acid; a quinic acid to malic acid ratio of not less than 1.0 and not more than 0.05% each of sorbitol and sucrose.

The following herbal preparation has been reported as the constituent of medicinal products on the market in the EU/EEA Member States (for further information see section 2 "Data on medicinal use"):

Dried cranberry juice (Denmark)

Dried, refined extract of cranberry juice (Austria, France, Netherland, Spain and United Kingdom)

- 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 *Vaccinii macrocarpi* fructus:

- Relevant articles and references retrieved from PubMed. Search term: Cranberry. Publication year: January 1990-July 2016. All in all 1095 publications were listed. A new search was done in September 2019.
- A part of the literature was provided by the Pharmatoka SAS and PhytoLab Companies in response to the call for scientific data in October 2015.
- Libraries: EMA library, library of the National Institute of Pharmacy and Nutrition, the national competent authority in Hungary.
- Textbooks, pharmacopoeias and monographs.

The abstracts of the references found were screened manually and all articles identified that could have a possible impact on the assessment report and monograph were included. This assessment report is based on the summary of the most relevant scientific literature.

2. Data on medicinal use

2.1. Information about products on the market

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

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Strength (where relevant)** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
dried cranberry juice	Herbal medicine for the prevention and treatment of mild, recurrent urinary tract infections	2 capsule three time daily "1 capsule contain: 405 mg juice, (as dried juice) from Vaccinium macrocarpon Aiton, syn Oxycoccus macrocarpos (Aiton) pers., fructus"	1996, Denmark, Iceland, MA
Dry extract; Extraction solvent: Ethanol 70% (v/v)	Traditional herbal medicinal product used to help prevent recurrent uncomplicated acute	One hard capsule contains 195-216 mg of extract (as dry extract, refined) from the juice of cranberry	UK (22.09.2016), TUR

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
	urinary tract infections (UTIs) such as cystitis in women only, based on traditional use only.	fruit (*Vaccinium macrocarpon* Ait. Fructus), corresponding to 36 mg of proanthocyanidins (PAC), calculated as PAC A2. At the first sign of any symptoms, take one capsule each day.	
Dry extract; Extraction solvent: Ethanol 70% (v/v)	A traditional herbal medicine to prevent the recurrence of acute uncomplicated lower urinary tract infections (cystitis) based solely on traditional use.	One hard capsule contains 195-216 mg of extract (as dry extract, refined) from the juice of cranberry fruit (Vaccinium macrocarpon Ait. Fructus), corresponding to 36 mg of proanthocyanidins (PAC), calculated as PAC A2. At the first sign of any symptoms, take one capsule each day.	Spain (14/12/2017), TUR
Dry refined extract DER: 250:1	A traditional herbal medicinal product for the prevention of recurrent urinary tract infection in healthy non- pregnant adult women	Each capsule contains 195-216 mg extract (dry, refined) from the juice of the cranberry fruits (*Vaccinium macrocarpon Ait. Fructus*), corresponding to 36 – 40 g cranberry juice and 36 mg proanthocyanidine (PAC), calculated as PAC A2)	NL (03/07/2019), TUR

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

Not applicable

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

There are plethora of products mainly in the area of food supplements in the EU/EEA.

Many countries indicated (AT, IE, LV, NL, SE) that there are products on the market as food and as food-supplement.

Cranberry juice is predominantly made from frozen fruits either by pressing or extracting in water. Each production batch is tested for brix, titratable acidity, haze, and colour; Cranberry Juice Cocktail® (Ocean Spray) is typically used as the reference point. Cranberry juice concentrate is prepared by hot mash depectinization of fresh or frozen cranberries (Upton & Brendler, 2016).

Cranberry Juice Cocktail is typically composed of 27% juice (7.5° Bx) with water and sweetener added. This beverage can be made either from juice, juice concentrate, or a blend of the two (Upton & Brendler, 2016).

In 1993 Craisins® Sweetened Dried Cranberries of the Company were introduced, becoming increasingly popular in supermarkets and bakeries all over the world.

Czech Republic

Food-supplements

Cys Control ®, Arkopharma

Powder for solution in bags containing 0.648 g of concentrated extract per 1 bag corresponding to 18 mg of procyanidins and 9.05 mg of ursolic acid to be used at the first signs of urinary discomfort, to alleviate symptoms and promote urinary comfort, while preserving the balance of the microbiota.

Daily dose 2 bags, 1 bag in the morning, 1 bag in the evening; children above 3 years of age ½ bag twice daily, recommended duration of use 20 days, drink more than 1.5 l of fluids

Apo-brusinky, ProPharma-Produkt

One capsule contains 500 mg of CranRich™ extract (36:1) 500 mg equivalent to 18 g of fresh fruits.

The product is recommended to support urinary tract health.

Dosage: adults 1-2 capsules once daily, children above 3 years of age 1 capsule daily.

Max brusinky, Swiss Herbal Remedies Ltd.

Tablets containing Cran Max® extract (34:1) 250 mg/tbl equivalent to 8.5 g of whole fruits

Daily dose 2 tablets, children above 3 years of age 1 tablet. For maximum effect, use 4-8 weeks. 2 tablets are equivalent to 0.25 l of 100% cranberry concentrate. It helps to promote Urinary Tract health.

Max Cranberry (Swiss Herbal Remedies Ltd., Canada)

Tablets containing 250 mg extract Cran Max® (DER= 34:1). Declared content of at least 5% anthocyan. Cran-Max® has been clinically shown to help promote a healthy Urinary Tract.*. The recommended dose is two tablets a day for at least four weeks. Use is recommended for children from three years (1 tablet daily).

Cran Urin™ Barnys®

Urinary and genital dietary supplements. Liquid containing CranRichPAC™ extract 400 mg/10 ml. Daily dose 20 ml (10 ml twice daily, children above 3 years of age 5 ml twice daily)

Urinal, Walmark

The product helps maintain urinary tract health before or at the time you feel signs of infection. Capsules containing concentrated dried juice 200 mg/cps. Dosage up to 6 capsules per day

Urinal® Syrup (Idelyn-Walmark)

The product cleanses the body, removes waste material and removes toxins. 5ml (1 teaspoon) NutriCran (dry cranberry extract) 500 mg. A maximum daily dose (15 ml) corresponds to a minimum of 37 500 mg of cranberry fruit. It is suitable for children from 1 year old, teenagers and adults.

Ireland, UK

Cran Med Forte Prevention (Boots) Medical device

Cranberry extract: A-type proanthocyanidin (PAC A) content per capsule is 36 mg based on the BL-DMAC method. **Prevents recurrent urinary tract infections / cystitis caused by *E. coli*.** Dosage and use, adults and children aged 12 and older: Once a day 1 capsule. Take the capsules with a large glass of water (250 ml). The product should not be used for a longer than 28 days in each period. Intake can be resumed after 2 days.

Assessor's comment:

In the UK cranberry products (juice, tablets, capsules) are not regulated and the concentration of active ingredients is not known. Concentrations may also fluctuate between batches of the same product.

Hungary

Walurinal Medical, Medical device

The product is recommended for cystitis and other lower urinary tract to treat infections and their recurrence whether of bacterial origin or not fungal (Candida or other) infection consists of in their background.1 tablet contains 120 mg dry cranberry extract. Dosage: 1 tablet daily

Germany

proSan Cranberry-36 PAC

For healthy bladder mucous membranes and a strong immune system.

Only 1 capsule a day - for prevention and long-term administration

36 mg Cranberry -PAC - optimally effective amount proven in studies

+ Vitamins A, B2, B3 and biotin for healthy mucous membranes

+ Vitamin C, zinc and selenium for a strong immune system

Avitale Cranberry Kapseln (Mikro-Shop Handels GmbH)

Food supplement with 400 mg cranberry powder AV36®. 2 capsules contain 800 mg cranberry concentrate powder AV36®. According to the current production analysis, this corresponds to at least 36 mg proanthocyanins. Cranberries or their ingredients can be a valuable support for general well-being, because the fruits contain many phytochemicals such as flavonoids, polyphenols and above all proanthocyanins.

Sweden

I SAY: Urinary Tract Infection (UTI) capsules

Medical device for treatment and prevention of Urinary Tract Infections. The composition of the capsules is: Cranberry Active™, Potato Starch, Hydroxypropylmethylcellulose. Cranberry Active™ is a patented extract (a concentrate) of cranberries. The recommended dose is from the age of 12 is: for treatment: 2 capsules daily for a period of 15 days, for prevention: 1 daily capsule per day with a glass of water.

Assessor's comment:

The 'Information on other products marketed in the EU/EEA' is not exhaustive. The information included was provided by the MS upon a request for information that was sent in 2015. Importantly, in August 2017 the COMMISSION IMPLEMENTING DECISION (EU) 2017/1445 of 8 August 2017 was adopted. The Commission decision stated that the group of products whose principal intended action, depending on proanthocyanidins (PAC) present in cranberry extract, is to prevent or treat cystitis, are not medical devices within the meaning of Article 1 (2) (a) of the Medical Devices Directive. Metabolites of PAC and other constituents of cranberry exhibit most probably a pharmacological activity.

Furthermore, there is no authorised health claim for food supplements containing cranberry or cranberry constituents.

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

There are many food supplements on the market outside the EU/EEA worldwide (Argentina, Australia, Canada, Chile, Singapore, USA) (Sweetman 2011).

In 1997, cranberry was in the 'top ten' of remedies sold by herbalists in the US. The usual preparations include fresh whole berries, gelatinized products, juices (these are usually 10–25% v/v pure juice), juice concentrate powder and capsules. Pure juice is too acidic (pH 2.5) and unpalatable, even when diluted with traditional sweetening vehicles (Guay, 2009; Siciliano, 1996).

In addition to the herbal preparation reported as constituents of medicinal products, there is a broad range of dietary cranberry products on the market worldwide, including liquid cranberry juice products of various dilutions, both sweetened and unsweetened, and cranberry juice concentrates in liquid and dry (powdered, flaked, or granulated) forms, the latter available in capsules, tablets, and teabag-infusion products, as well as products made from the pomace (micronized dried cranberry pulp and skins, seeds, stems; also known as press cake). The composition of the bioactive compounds differs markedly between products and is affected by processing method (Upton & Brendler, 2016).

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

Native Americans had been consuming wild cranberries for centuries as part of a foodstuff called pemmican, a combination of crushed berries, fat, and dried meat (Siciliano, 1996). There is no recorded medicinal use of cranberry among tribes of Massachusetts, one of the primary growing areas

for native cranberry populations. Other American Indian tribes (Canada) steeped cranberry branches to make a tea for pleurisy. The fruits (sometimes unripe) were used medicinally as a poultice for wounds, and, mixed with cornmeal, as a drawing poultice for blood poisoning. First Nation peoples in Quebec crushed and applied cranberries to facilitate the healing of cancerous sores (decubitus ulcers) (Upton & Brentler, 2016).

Therapeutic applications of cranberries documented during the 17th century included the relief of blood disorders, stomach ailments, liver problems, fevers, vomiting, appetite loss, scurvy, and cancer. New England folk medicine practitioners used boiled cranberries and seal oil to reduce the severity of gall bladder attacks (Siciliano, 1996; Upton & Brendler, 2016).

Cranberries do not appear in many of the American materia medicas of the 18th century.

In the early 1800s cranberries were mentioned as mild laxative, refrigerant, antiseptic, diuretic, anti-pyretic, mild astringent and antiscorbutic. It was also used as a poultice of raw cranberry fruits as a treatment for cancerous tumours and erysipelas (Upton & Brendler, 2016; Erichsen-Brown, 1989). In 1905 the use of the fruit in domestic practice as a poultice for erysipelas, inflammatory swellings, swollen glands, indolent and malignant ulcers, tonsillitis, and for boils on the tip of the nose was noted (Upton & Brendler, 2016). It is also recorded that cooked cranberries were eaten to cure haemorrhoids (Erichsen-Brown, 1979).

Lots of articles referred to the traditional use of cranberry juice for preventing and treating urinary tract infection which investigated the effect of cranberry on pH of the urine suggesting the benzoic acid (Blatherwick, 1914, Blatherwick *et al*., 1923) or the quinic acid (Fellers 1933, Kahn *et al* 1967, Kinney 1979) caused large amounts of hippuric acid to be excreted in the urine (Bodel *et al* 1959), which then acted as an antibacterial agent.

Sobota (1984) also referred to the traditional use when investigated other possible mechanism of action, *antiadherence activity in the urine,* behind the traditional use.

Some early observations derived from the general practice or clinical studies as well.

Moen 1962 found cranberry juice useful for the treatment of urinary tract infection. Sternlieb 1963 described that an 8 [=227 ml]-ounce glass four times daily for several days followed by 1 such glassful twice daily is valuable adjunctive therapy and prophylaxis … in patients with certain urinary-tract infections."

Papas *et al* 1966 found in a clinical study that after three weeks of treatment with 16 ounces [455 ml] of cranberry juice per day, 53 per cent of the patients with acute urinary tract infection had a positive clinical response.

In 1994 a randomised double blind, placebo controlled trial was performed to determine the effect of a daily intake of 300 ml cranberry juice cocktail on bacteriuria and pyuria in 153 elderly women (Avorn 1994).

Since than lots of clinical studies have been performed with different preparations (see 4.2 Clinical efficacy) and the use of V. macrocarpon juice to treat UTI is reported in different manuals of phytotherapy.

Manuals of phytotherapy:

Cranberry Liquid preparation was included in the 19th edition of the United States Pharmacopeia-National Formulary (2000).

Health Canada has published a **monograph** on cranberry and on dried cranberry juice as a guide to industry for the preparation of Product Licence Applications (PLAs) and labels for natural health product market authorization (Health Canada, 2011, 2018).

Proper name(s): *Vaccinium macrocarpon* Aiton (Ericaceae) (USDA 2010; McGuffin *et al*. 2000)

Common name(s):

- Cranberry (McGuffin *et al*. 2000; Wiersema and Léon 1999)

- American cranberry (McGuffin *et al*. 2000; Wiersema and Léon 1999)

- Large cranberry (McGuffin *et al*. 2000; Wiersema and Léon 1999)

Dried cranberry juice (Jepson and Craig 2008; Mills and Bone 2005; Stothers 2002; Upton 2002; Siciliano 1996)

Source material(s): Fruit (Jepson and Craig 2008; Mills and Bone 2005; Stothers 2002; Upton 2002; Siciliano 1996)

The monograph includes three indications:

Adults 18 years and older (2018)

1. (Traditionally) used in Herbal Medicine to help prevent (recurrent) urinary tract infections (UTIs) (Barnes *et al*. 2007; Bruyère 2006; Blumental *et al*. 2003; Bodel *et al*.1959),

2. (Used in Herbal Medicine to) help(s) prevents recurrent urinary tract infections (UTIs) in women (Jepson and Craig 2008; Mills and Bone 2005; Stothers 2002; Walker *et al*. 1997; Avorn *et al*. 1994).

3. Provides antioxidants for the maintenance of good health (Valentenova *et al*. 2007; Ruel *et al*. 2005; Upton 2002)

Dosage:

1. (Recurrent) urinary tract infection:

Preparations equivalent to 90-950 ml fruit juice, per day (Blumenthal *et al*. 2003; Stothers 2002; Avorn *et al*. 1994)).

Preparations equivalent to 10-30 g fresh fruit, per day (Mills and Bone 2005; Upton 2002; Walker *et al*. 1997).

400-1200 milligrams of dried fruit juice (powdered) per day (Mills and Bone 2005; Upton 2002; Walker *et al*. 1997).

Duration of Use: Use for a minimum of 4 weeks to see beneficial effects ((Jepson and Craig 2008; Blumenthal *et al*. 2003; Walker *et al*. 1997; Avorn *et al*. 1994).

2. Antioxidant:

Preparations providing an equivalent of up to 950 ml fruit juice, per day (Ruel *et al*. 2005; Blumenthal *et al*. 2003; Stothers 2002)

Not exceed 950 mililiters of fruit juice, per a day (Ruel *et al*. 2005; Blumenthal *et al*. 2003; Stothers 2002)

Up to 1200 mg dried fruit juice, per day (Valentenova *et al*. 2007; Upton 2002)

Preparations providing an equivalent of up to 30 g fresh fruit, per day (Valentenova *et al*. 2007; Upton 2002).

Not to exceed 30 grams of fresh fruit, per day (Valentenova *et al*. 2007; Upton 2002)

Duration of use: No statement required (2011).

American Herbal Pharmacopoeia (Upton-Brendler 2016) cranberry fruit (*Vaccinium macrocarpon* Aiton, Ericaceae) lists the following indications:

Indications Supported by Clinical Trials: Beneficial for the prevention of recurrent urinary tract infections (UTIs); helps reduce the adhesion of certain E. coli bacteria to urinary tract walls.

Indications Supported by Modern Research: Prophylactic against recurrent urinary tract infections; prevents P-fimbriated and type 1-fimbriated uropathogenic strains of E. coli from adhering to mucosal cells in the urinary tract; reduces bacterial biofilm formation in the urinary tract, anti-inflammatory, antioxidant.

Commercial juice drink (27% cranberry): 300–500 mL/ day

Dried cranberries: 30–40 g/day

Cranberry sauce (whole or jellied): 45 g/day

Encapsulated powder:

Juice-based 200–3500 mg/day

Whole berry or skin-based press cake: 500–1000 mg/day

Cranberry juice is included in many pharmacognostical texts and handbooks, e.g. ESCOP 2009, WHO 2009; Martindale (Parfitt, 1999, Sweetman 2011); Potter's (Williamson, 2003); Bartram, 1995; Tyler, 1994).

In the literature sources the herbal preparation is mainly recommended for the treatment and prevention of recurrent urinary tract disorders. This indication and posologies documented in these sources are derived from clinical studies/food supplements.

Based on American herbal pharmacopeia (Upton 2002), the WHO (2009) recommends for the prevention of urinary tract infections (UTIs) in adults daily dose of 10–100 ml cranberry juice; for the treatment of UTIs in adults the daily dosage range is 120–1600 ml or equivalent.

Capsules containing a concentrated cranberry extract: 1–6 capsules daily, equivalent to 3 fluid ounces (90 ml) cranberry juice or 400–450 mg cranberry solids

ESCOP (2009) recommends 300-750 ml per a day of a liquid preparation containing 25-100% of cranberry juice, divided into 2-3 portions; 200-500 mg of dry extract or juice concentrate twice a daily; other equivalent preparations (McMurdo *et al*. 2009; Slothers 2002; Walker *et al*. 1997; Avon *et al*. 1994; Havernkorn *et al* 1994) for prevention of urinary tract infection (McMurdo *et al*. 2009; Slothers 2002; Kontiokari *et al* 2001; Walker *et al*. 1997; Avon *et al*. 1994; Havernkorn *et al* 1994; Hess et al. 2008, Jepson *et al*. 2008).

UTIs Guidelines

Cranberry can also be found in guidelines or in national information brochures for patients. In the brochures published by U.S. Department of Health and Human Services & National Institutes of Health for prevention of UTI, drinking cranberry juice is advised (2005, 2007, 2010).

In the national clinical guideline for management of suspected bacterial UTI, published by the Scottish intercollegiate guidelines network, it is advised for women with recurrent UTI to consider using

cranberry products to reduce the frequency of recurrence (Scottish Intercollegiate Guidelines Network, 2012).

According to the Guidelines on Urological infections the daily consumption of cranberry products, giving a minimum of 36 mg/day proanthocyanindin A is recommended for the prevention of UTIs (European Association of Urology, Grabe *et al* 2010, 2013). In the updated versions of the guideline in 2015, 2020, it is mentioned that limited studies have suggested that cranberry is useful in reducing the rate of lower UTIs in women [Kontiokari *et al*. 2001, Slothers L 2002]. However, a meta-analysis including 24 studies and comprising 4,473 participants concluded that cranberry products did not significantly reduce the occurrence of symptomatic UTI for women with recurrent UTI [Jepson *et al*. 2008]. Due to these contradictory results, the European Association of Urology does not recommend daily consumption of cranberry products as for prophylaxis for UTI. (European Association of Urology, Grabe at al 2015, Bonkat *et al* 2018, 2020).

The guidelines of the American Urological Association, the Canadian Urological Association and the Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction, recommend cranberries despite a fairly low level of evidence for UTI with a grade C of recommendation Anger *et al* 2019).

On July 21, 2020, Food and Drug Administration (FDA) announced qualified health claim for certain cranberry products and a reduced risk of recurrent urinary tract infection (UTI) in healthy women. According to the FDA, there is limited and inconsistent credible scientific evidence to support a qualified health claim for the consumption of cranberry juice beverages and limited credible scientific evidence to support a qualified health claim for the consumption of cranberry dietary supplements and a reduced risk of recurrent UTI in healthy women.

Table 2: Overview of historical data

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form, Strength (where relevant), Posology, Duration of use**	**Reference**
Cranberry juice	Folklore Medicine for the relief of the frequent and ancient complaint of dysuria. For the treatment of urinary tract infections.	Dosage: 2 times daily 6 oz cranberry juice [= 2 x 170 ml = 341 ml]	Moen 1962
Cranberry juice	Preventing and treating some renal problems (such as lithiasis and certain urinary tract infection.	usual dosage: 12-32 ounces (341-909 ml) daily 8 [227 ml]-ounce glass four times daily for several days followed by 1 such glassful twice daily	Sternlieb 1963
Cranberry Juice Cocktail (1961)	To reduce the risk of urinary tract infection in women.	The serving size is 8fl oz (240 ml) of the cocktail and 40 g of Craisins® Sweetened Dried Cranberries and the	EFSA (2009).

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form, Strength (where relevant), Posology, Duration of use	Reference
Craisins® Sweetened Dried Cranberries (1993)		regular consumption is 2 serving per day	
Cranberry juice cocktail (about 1/3 of which is pure juice)	Treatment and prevention of UTIs	90 ml for prevention, 360-960 ml daily for treatment.	Tyler, 1994
capsules containing dried cranberry powder		6 capsules of cranberry powder equivalent to 90 ml of cocktail	
fresh/frozen cranberries		45 g fresh/frozen cranberries equivalent to 90 ml of cocktail	
Cranberry juice concentrate: 12.1-140 mg (equal to 1680 mg of fresh cranberries)	UTIs	2 capsules daily	Bartram, 1995
Fresh juice	For mild UTIs	15fl oz (440 ml) daily	Bartram, 1995
Juice	Treatment and prevention of UTIs	Up to 960 ml daily	Williamson, 2003 (Potter's)
Commercial juice	Reduce bacteruria	75-300 ml/day	Mills & Bone, 2005
Dry concentrate (25:1)		400-800 mg/day	
Fresh fruit or equivalent in tablet or capsule form		10-20 g/day	
Cranberry liquid preparation containing 25-100% of cranberry juice	Prevention of UTIs	300-750 ml/day divided into 2 or 3 portions	ESCOP 2009
Cranberry dry extract or juice concentrate		200-500 mg twice daily	
Cranberry juice		15 ml/kg bw	
Dried fruit juice	Traditionally) used in Herbal Medicine to help prevent (recurrent) UTIs	400-1200 mg per day Use for a minimum of 4 weeks	Health Canada, 2011, 2018
Dried fruit juice	(Used in Herbal Medicine to)	400-1200 mg per day. Use for a minimum of 4 weeks	

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form, Strength (where relevant), Posology, Duration of use	Reference
	help(s) prevent recurrent UTIs in women		
Dried fruit juice	Provides antioxidants for the maintenance of good health	up to 1200 mg per day	
Cranberry capsules	Prevention of UTIs	Women with recurrent UTI to consider using cranberry products. Cranberry capsules may be more convenient than juice and that high strength capsules may be most effective.	Scottish Intercollegiate Guidelines Network, 2012
Cranberry products	Prevention of UTIs	minimum of 36 mg/day proanthocyanindin A	European Association of Urology (2010, 2013)
Cranberry products	Cranberry products	Commercial juice drink (27% cranberry): 300–500 mL/ day Dried cranberries: 30–40 g/day Cranberry sauce (whole or jellied): 45 g/day Encapsulated powder: Juice-based 200–3500 mg/day Whole berry or skin-based press cake: 500–1000 mg/day	American Herbal Pharmacopoeia (Upton-Brendler 2016

2.3. Overall conclusions on medicinal use

Table 3a: Overview of evidence on period of medicinal use for the relief of symptoms of recurrent urinary tract infections

Herbal preparation Pharmaceutical form	**Indication**	**Strength Posology**	**Period of medicinal use**
Cranberry juice	Treatment of UTIs	Two times daily 60[1] ml juice	Moen 1962
Cranberry juice	treatment of urinary tract infection	80 ml juice 4 times daily[1)]	Sternlieb, 1963
Cranberry juice	Treatment of UTIs	120-320 ml daily for treatment	Tyler, 1994
405 mg dried refined cranberry juice	Herbal medicine for the prevention and treatment of mild, recurrent urinary tract infections	2 capsule three times daily This corresponds to drinking 1 1/2 litres of diluted cranberry juice (Jeno-Pharm 2011a)	1996, Denmark, Iceland, MA

Table 3b: Overview of evidence on period of medicinal use for **prevention** of urinary tract infections

Herbal preparation Pharmaceutical form	**Indication**	**Strength Posology**	**Period of medicinal use**
Cranberry juice	Prevention of UTIs	30 ml daily	Tyler, 1994
Cranberry juice	Reduce the risk of urinary tract infection in women	80 ml 2 times daily	EFSA 2009
405 mg dried refined cranberry juice	Herbal medicine for the prevention and treatment of mild, recurrent	2 capsule three times daily This corresponds to drinking 1 1/2 litres	1996, Denmark, Iceland, MA

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
	urinary tract infections	of diluted cranberry juice (Jeno-Pharm 2011a)	

Well-established use:

The medicinal product containing 405 mg concentrated juice has been authorised in Denmark and Iceland in 1996. The clinical efficacy and the requirements for the establishment of a well-established use monograph in the clamed indication are assessed in chapter 4 'Clinical data'.

Traditional use:

Fifteen years of use of Cranberry juice in the European Union is considered demonstrated based literature data and on the fact that a medicinal product with concentrated juice has been authorised in Denmark and Iceland in 1996. Thirty years of medical use for prevention and relief of symptoms of recurrent UTI is documented in several sources. (Bodel *et al* 1959, Moen DV 1962, Papas 1966, Kahn *et al*., 1967, Kinney *et al*., 1978, Sobota 1984, Sternlieb 1963 Gibson *et al*., 1991 and Tyler 1994).Taking into account all of these historical data on the traditional use of cranberry from North America, it can be concluded, that the 30 years of requirement in medicinal use is fulfilled for the Cranberry juice.

In conclusion, the following herbal preparation, indication and posology fulfil the criteria for traditional medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA:

Expressed juice from the fresh fruit - (DER 1: 0.6-0.9)

Assessor's comment:

1 litre juice can be pressed from 1500 g of cranberry (Bodel et al 1959). The obtained yield is approx. 58 - 66 %, using frozen berries approx. 70 %, using pectolyticenzymes approx. 86 – 90 % (Weiss 1977). DER 1: 0.60-0.90 includes both references.

Therapeutic indication:

Lots of articles (Bodel et al 1959, Moen DV 1962, Sternlieb 1963, Papas 1966, Kahn et al., 1967, Kinney et al., 1978, Sobota 1984 and Tyler 1994) refer to the traditional use of cranberry for prevention and treatment of UTI, so the following indication is recommended in the monograph:

Indication 1)

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.

Indication 2)

Traditional herbal medicinal product used for prevention of recurrent uncomplicated lower urinary tract infections in women, after serious conditions have been excluded by a medical doctor.

The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Posology and method of administration

Indication 1)

30-80 ml daily 2-4 times daily

Indication 2)

15-80 ml twice daily

Assessor's comment:

The liquid and solid cranberry preparations that are recommend are commercial products, variously, and often inadequately described as cranberry juice, cranberry juice cocktail concentrated juice, dried juice concentrates, dried refined juice etc.

The posology of the traditional medicinal products registered in EU Member States are comparable with the posology recommended by Tyler (1994).

The product that is authorized in Denmark and Iceland is not included in the monograph because the DER of the refined extract is not known. The dry refined extract which is registered as a traditional medicinal product in several Member States is also not included in the monograph due the fact that 15/30 years of use of this refined extract are not yet demonstrated. However, based on the DER provided by NL, 195-216 mg of the extract corresponds with 49-54g fresh cranberries. According to Tyler (1994) 45 g fresh/frozen cranberries corresponds with 30 ml of juice. Hence, the posology the range is the of the monograph

Duration of use

Indication 1)

If the symptoms persist for more than 4 days during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Indication 2)

No restriction.

If the patient experience symptoms of urinary tract infection during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

3. Non-Clinical Data

Many pharmacological studies have been conducted with extracts and isolated constituents of *Vaccinium macrocarpon in vivo* and *in vitro*. A systematic review of all of these studies will not be attempted here; rather a selection of studies with emphasis on studies with relevance for the plausibility of traditional use is presented. Furthermore, the EMA Committee for Medicinal Products for Human use (CHMP) evaluation on the principal mode of action of proanthocyanidins intended to be used for prevention and treatment of urinary tract infections (EMA/427414/2016) has been taken into account.

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

3.1.1. Primary pharmacodynamics

Urinary tract infections (UTIs) refer to the presence of microorganisms in the bladder, prostate, collecting system, or kidney. UTIs are extremely prevalent, especially in females, the elderly, and infants. Certain groups, especially women, are more prone to repeated infections. UTIs are usually caused by Gram-negative bacteria, especially Escherichia coli. *E. coli* remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections and is the most prevalent pathogen associated with UTIs in young children (Liu, 2006). Hence the selection of studies below are focused on research regarding *E. coli*.

UTI

In vitro+in vivo

12 dogs with a history of recurrent UTI received an antimicrobial (n=6) or **powdered cranberry extract** (DER and extraction solvent unknown) (n=6) orally for 6 months (1 g for dogs < 25 kg and 2 g for dogs ≥ 25 kg). Dogs were monitored for a UTI. For the *in vitro* experiment, cranberry extract was orally administered to 6 dogs for 60 days. Voided urine samples were collected from each dog before and 30 and 60 days after onset of extract administration. Urine was evaluated by use of a bacteriostasis assay. None of the 12 dogs developed a UTI. The bacteriostasis assay revealed no zone of inhibition for any urine samples. Bacterial adhesion was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results for urine samples obtained before extract administration. Microscopic examination revealed that bacterial adherence to MDCK cells was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results after culture with urine samples obtained before extract administration (Chou *et al.,* 2016).

In an experimental model of urinary tract infection (UTI) mice were inoculated transurethrally with 50µl of bacterial suspension containing ~ $5x10^8$ CFUs (Jensen *et al*. 2017). The bacterial counts of infected bladders in each group were compared to the control groups. After inoculation, the 7 day treatment was performed with experimental (n=45) and control groups (n=100). Experimental groups received as a drinking fluid: Group 1 – **cranberry juice cocktail** (n=10), Group 2 – **fresh cranberry juice** (n=35); control groups received as a drinking fluid: Group 3 – water (n=47), Group 4 – hydrophilic fraction (n=17), Group 5 – mixture of organic acids (n= 36). Significantly reduced bacterial counts were found in the bladder ($p< 0.01$) of mice drinking fresh cranberry juice. Treatment with both commercially available Cranberry Juice Cocktail and fresh cranberry juice reduced the CFU in the bladder by 65% ($P < 0.01$) and 47% ($P < 0.01$), respectively. Also treatment with the hydrophilic fraction of cranberry juice decreased CFU in the bladder by 44% ($P < 0.05$; Table 1) whereas treatment with the ethanolic fraction of cranberry juice had no effect. The four organic acids (quinic, malic, shikimic, and citric acid) administered together decreased bacterial count in the bladder ($p< 0.001$), and the combination of malic plus citric acid ($p< 0.01$) and malic plus quinic acid ($p< 0.05$).

Anti-adherence activity

Ex vivo/in vitro

Cranberry cocktail was given to mice in the place of their normal water supply for a period of 14 days. Urine collected from these mice inhibited adherence of *E. coli* to uroepithelial cells by approximately 80% ($p<0.01$) (Sobota, 1984).

Groups of mice were given either water (control), **cranberry juice cocktail** (CJC), **cranberry proanthocyanidins (50 mg/300 ml water),** or cranberry proanthocyanidins (500 mg/300 ml water) as a drinking source. Urine was collected every 5 days for 30 days with one baseline collection before treatments began. Urine was tested for the ability to prevent P-fimbriated *E. coli* anti-adherence activity to uroepithelial cell surfaces. All baseline and control urine samples were negative for antiadherence activity, whereas the urine from the CJC and both cranberry proanthocyanidin treatments exhibited positive anti-adherence activity at certain times during the test period. According to the authors, this outcome indicates that cranberry proanthocyanidins may be absorbed following ingestion and active metabolites may be reaching the urine to elicit the positive bacterial anti-adherence effect (Howell *et al.,* 2001).

The activity of an Cranberry extract containing 118 mg of PACs per dose on *Escherichia coli* adherence to bladder epithelial cells has been studied *in vitro*. The rats received the extract per orem, and urine from each animal was collected during the following 16 hours and preincubated with *E. coli*. Subsequently, bacteria were incubated with T24 cells. Urine samples from rats taking the extract powder for oral suspension and tablets (118 mg PACs/animal) showed an important inhibition of *E. coli* adherence (83% and 52%respectively). The lower dose of 59 mg PACs/animal also showed marked inhibition of *E. coli* adherence (29% after tablets intake and 40% for powder). *In vitro*, the extract showed inhibition of bacterial adherence in all tested concentrations: 5, 25 and 75 PACs mg/ml, diminishing the number of bacteria adhered to epithelial cells by 25%, 36% and 34% respectively (Risco *et al.,* 2010).

Rats (n=12/group) were grouped into control group, 25 % Juice, 100 % Juice groups, which were tube-fed with 1 ml H_2O and 25 % cranberry juice concentrate or 1 ml 100 % cranberry juice concentrate three times per day, respectively. Their urine has been shown to decrease the capability of *E. coli* in hemagglutination, urothelium adhesion, nematode killing, and biofilm formation. All these changes occurred after *E. coli* was incubated in cranberry metabolite-containing urine, defined as urine opsonization. Urine opsonization of *E. coli* resulted in 40.9% (p=0.0038) decrease in hemagglutination ability, 66.7% (p=0.0181) decrease in urothelium adhesiveness, 16.7% (p=0.0004) increase in the 50% lethal time in killing nematodes, and 53.9% ($p=5.9\times10^{-4}$) decrease in biofilm formation (Chen *et al.,* 2013).

Antiadhesive activity of cranberry against uropathogenic Escherichia coli was tested in healthy Sprague-Dawley rats (Peron *et al*. 2017). Two experiments were performed. In the 1 experiment animals received every day for 35 days a „standardised" **cranberry extract** (100 mg/kg of extract, containing 15% of total PACs), to mimic a prolonged treatment of cranberry. The 24-h urinary outputs were collected weekly at days 0, 7, 14, 21, and 35 during the study. The urine samples were subjected to UPLC−electrospray ionization-quadrupole time-of-flight (ESIQTOF) analysis. Microbial PAC metabolites, such as valeric acid and valerolactone derivatives, corelated with cranberry intake were found. An increased urinary excretion of glucuronidated metabolites was also seen. In the second experiment a single dose of cranberry (oral gavage of 100 mg/ kg of extract containing 15% of total PACs) was administered to rats (n = 6) and the changes of urinary composition at 2, 4, 8, and 24 h after extract administration were observed. Antiadhesive properties of all the urine samples were tested against Escherichia coli. The highest activity showed the 8 h samples. However, the highest amounts of PAC-A2, on the order of ng/mL, were found in the samples collected after 4 h. These results show that the antiadhesive activity against bacteria seen after cranberry intake is corresponding to PAC-A metabolites rather than to a direct PAC-A effect, as the PAC-A levels in urine were lower than those reported as pharmacologically active in the literature.

Adult female pigs were fed spray-dried **cranberry powder** (5 g/kg/day), and urine was collected via catheter. Urine fractions were tested for antiadhesion activity using a human red blood cell (A+) anti-

hemagglutination assay with uropathogenic P-fimbriated *E. coli* (Coleman *et al*., 2019). In urine fractions a complex series of oligosaccharides but not proanthocyanidins were found, and a single representative arabinoxyloglucan octasaccharide was isolated. Cranberry material contained a similar complex series of arabinoxyloglucan oligosaccharides exhibited antiadhesion properties in preliminary testing. The results indicate that oligosaccharides may contribute to the antiadhesion properties of urine after cranberry oral intake.

In vitro

Cranberry juice/juice cocktail/juice extract

Boland (2016) determined the ability of **cranberry juice** and various other cranberry products to inhibit P-fimbriated *E. coli* from binding to human erythrocytes as measured by hemagglutination (HA). The results of this study demonstrated that cranberry juice can block P-fimbriae mediated binding of *E. coli* to human cells when incorporated into the bacterial growth media and also directly interferes with P-fimbriae binding to their ligand. Comparison of cranberry products revealed significant differences among individual products to block P-fimbriae mediated host cell binding. The efficacy of the cranberry products' ability to directly inhibit *E. coli* DS17 adherence varied widely. Cranberry juice was effective at disrupting bacterial adherence. Preincubation of the bacteria with the cranberry products revealed further differences in their ability to inhibit HA in a time-dependent manner.

The effect of **cranberry juice cocktail and juice** on the adherence of *Escherichia coli* expressing surface lectins of defined sugar specificity to yeasts, tissue culture cells, erythrocytes, and mouse peritoneal macrophages was examined. Cranberry juice cocktail inhibited the adherence of urinary isolates expressing type 1 fimbriae (mannose specific) and P fimbriae [specific for 0-D-Gal(1-+4)-1-D-Gal [Gal=galactose]) but had no effect on a diarrheal isolate expressing a CFA/I adhesion (CFA=Colonization factor antigen). The cocktail also inhibited yeast agglutination by purified type 1 fimbriae. The inhibitory activity for type 1 fimbriated *E. coli* was dialyzable and could be ascribed to the fructose present. The inhibitory activity for the P fimbriated bacteria was non-dialyzable and was detected only after pre-incubation of the bacteria with the cocktail, suggesting that high molecular weight constituents are involved. Cranberry juice, orange juice, and pineapple juice also inhibited adherence of type 1 fimbriated *E. coli*, most likely because of their fructose content. However, the two latter juices did not inhibit the P fimbriated bacteria. It was concluded that cranberry juice contains at least two inhibitors of lectin-mediated adherence of uropathogens to eukaryotic cells (Zafriri *et al.*, 1989).

Liquid cranberry concentrate without sugars or preservatives (pH 2.31) at pH 7.0 was added to CFA medium to a final concentration of 25%. *E. coli* strains JR1 and DS17 were plated on this medium with a plain CFA control and incubated at 37°C. It was concluded, that cranberry juice irreversibly inhibited P-fimbriae. Additionally, inhibited bacteria showed cellular elongation. Electron micrographic evidence suggested that cranberry juice acts on the cell wall preventing proper attachment of the fimbrial subunits or as a genetic control preventing the expression of normal fimbrial subunits or both (Ahuja *et al.*, 1998).

Experiments were conducted to investigate the molecular-scale effects of **cranberry juice cocktail** (CJC) on two *E. coli* strains: HB101, which has no fimbriae, and the mutant HB101pDC1 which expresses P-fimbriae. Atomic force microscopy was used to investigate both bacterial surface characteristics and adhesion forces between a probe surface (silicon nitride) and the bacteria, providing a direct evaluation of bacterial adhesion and interaction forces. CJC affected bacterial surface polymer and adhesion behavior after a short exposure period (<3 h). CJC affected the P-fimbriated bacteria by decreasing the adhesion forces between the bacterium and tip and by altering the conformation of the surface macromolecules on *E. coli* HB101pDC1. The equilibrium length of P-

fimbriae on this bacterium decreased from approximately 148 to approximately 48 nm upon being exposed to cranberry juice. Highly acidic conditions were not necessary for the prevention of bacterial adhesion, since neutralization of cranberry juice solutions to pH=7.0 allowed us to observe differences in adhesion between the *E. coli* strains. The results demonstrated molecular-level changes in the surfaces of P-fimbriated *E. coli* upon exposure to neutralized cranberry juice (Liu *et al.*, 2006).

In a later study, using the same strains and herbal preparation, this research group showed, that CJC significantly decreases nanoscale adhesion forces between P-fimbriated *E. coli* and uroepithelial cells (Liu *et al.*, 2010).

A thermodynamic approach was also used by this group to examine the bacteria-uroepithelial cell interactions. P-fimbriated *E. coli* was able to form strong bonds with the Gal-Gal disaccharide receptor on uroepithelial cells, while non-fimbriated *E. coli* interactions were only non-specific. In the P-fimbriated *E. coli* –uroepithelial cell system Gibbs free energy of adhesion values increased as a function of increasing CJC concentration until the point was reached where adhesion became unfavorable. According to the authors, these results may suggest that cranberry juice disrupts bacterial ligand-UC receptor binding (Liu *et al.*, 2008).

Freeze-dried whole cranberry powder (9 mg proanthocyanidin per g) reduced the mean adherence of P-fimbriated *E. coli* isolate to vaginal epithelial cells from 18.6 to 1.8 bacteria per cell ($p<0.001$). Mean adherence of *E. coli* to primary cultured bladder epithelial cells was decreased by exposure to 50 µg/ml proanthocyanidin extract from 6.9 to 1.6 bacteria per cell ($p<0.001$). Inhibition of adherence of *E. coli* by proanthocyanidin extract occurred in linear, dose dependent fashion over a proanthocyanidin concentration range of 75 to 5 µg/ml (Gupta *et al.*, 2007).

In a later study where three proanthocyanidin (procyanidin) (PAC)-"standardised" cranberry extracts (PAC equivalent concentrations of 0–180 µg/ml) and commercial PAC A2 were tested. Extract A was produced using purification steps, extract B corresponded to a mix between extract A and dried concentrated cranberry juice. Extract C was a dried cranberry juice concentrated extract. Significant reduction of adhesion to urepithelial cells was observed: around 80% of inhibition of adhesion with the cranberry extracts at equivalent PAC concentration of 50 µg/ml. The effects of the different assayed extracts were not obviously different except for extract B, which inhibited approximately 55% of adhesion at an equivalent PAC concentration of 5 µg/ml (Ermel *et al.,* 2012).

Atomic force microscopy was used to probe the adhesion forces between *E. coli* (nonfimbriated strain HB101 and the P-fimbriated variant HB101pDC1) and a model surface (silicon nitride), to determine the effect of growth in cranberry products on bacterial adhesion. Growth of *E. coli* HB101pDC1 and HB101 in light cranberry juice cocktail (L-CJC) or PACs resulted in a decrease in adhesion forces with increasing number of cultures. In a macroscale bacteria-uroepithelial cell adhesion assay a decrease in bacterial attachment was observed for *E. coli* HB101pDC1 grown in L-CJC or PACs. This effect was reversible because bacteria that were regrown in cranberry-free medium regained their ability to attach to uroepithelial cells, and their adhesion forces reverted to the values observed in the control condition. Exposure to increasing concentrations of L-CJC resulted in a decrease of bacterial attachment to uroepithelial cells for the P-fimbriated strain after L-CJC treatment (27% by weight) and after PACs treatment (345.8 µg/ml). The concentration of cranberry products and the number of cultures the bacteria were exposed to cranberry determines how much the adhesion forces and attachment are altered (Pinzón-Arango *et al.,* 2009).

Under *in vitro* conditions a dose-dependent increase in bacterial adhesion was observed with proanthocyanidin-enriched cranberry *Vaccinium macrocarpon* (V.m.) extract (proanthocyanidin content = 21%). Confocal laser scanning microscopy and scanning electron microscopy proved that V.m. extract led to the formation of bacterial clusters on the outer plasma membrane of the host cells

without subsequent internalization. This agglomerating activity was not observed when a PAC-depleted extract (V.m. extract(≠PAC)) was used, which showed significant inhibition of bacterial adhesion in cases where type 1 fimbriae dominated and mannose-sensitive UPEC strain NU14 was used. V.m. extract(≠PAC) had no inhibitory activity against P- and F1C-fimbriae dominated strain 2980. Quantitative gene expression analysis indicated that PAC-containing as well as PAC-depleted cranberry extracts increased the fimH expression in NU14 as part of a feedback mechanism after blocking FimH. For strain 2980 the PAC-containing extract led to up-regulation of P- and F1C-fimbriae, whereas the PAC-depleted extract had no influence on gene expression. V.m. and V.m. extract(≠PAC) did not influence biofilm and curli formation in UPEC strains NU14 and 2980. These data lead to the conclusion that also proanthocyanidin-free cranberry extracts exert antiadhesive activity by interaction with mannose-sensitive type 1 fimbriae of UPEC (Rafsanjany 2015).

The purpose of the study performed by Scharf *et al*. (2018) was to investigate if cranberry fruit extract acts only via a single component or must be assessed as a multiactive-compound preparation. Additionally, it was aimed to check whether the extract, besides the Tamm-Horsfall protein (THP) induction in the kidney, also acts directly against uropathogenic E. coli (UPEC). Further on, a series of isolated natural products, related to the typical composition of cranberry extracts, was to be investigated under in vitro conditions to pinpoint defined natural products with antiadhesive effects against UPEC, followed by in silico calculations to predict potential antiadhesive compounds by use of a validated quantitative structure activity relationship model (QSAR). Urine samples from 16 volunteers treated with cranberry extract (extract-fruit-ratio = 25:1, proanthocyanidins (HPLC) > 2.7 %, p.o., 7 days, 900 mg/day) were used for ex vivo testing concerning influence on the bacterial transcriptome (Illumina RNA-seq) and interaction with the mannose binding domain of type-1 fimbriae. The results suggests that B-ring substituted flavones and flavonols from cranberry contribute to the antiadhesive activity against UPEC by inhibition of the FimH-mediated interaction with the host cell bladder epithelium.

Isolated compounds

Proanthocyanidins of *Vaccinium macrocarpon* (epicatechin trimers, tetramers and pentamers) inhibit adherence of uropathogenic isolates of P-fimbriated *Escherichia coli* bacteria to cellular surfaces containing α-gal(1-->4)β-gal receptor sequences similar to those on epithelial cells in the urinary tract (Foo, 2000a, 2000b).

Isolated A-type proanthocyanidins from cranberry juice cocktail elicited *in vitro* anti-adhesion activity at 60 µg/ml, the B-type proanthocyanidins from grape exhibited minor activity at 1200 µg/ml, while other B-type proanthocyanidins (from green tea, apple juice, dark chocolate) were not active. Hence, it was suggested that presence of the A-type linkage in cranberry proanthocyanidins may enhance *in vitro* bacterial anti-adhesionactivities (Howell *et al.*, 2005).

A purified proanthocyanidin was extracted from dried cranberry juice. Its effect on multi-drug resistant bacteria as well as quantification of anti-adherence bioactivity on human vaginal and bladder epithelial cells was appraised. Inhibition of adherence to an extent of about 70% with multi-drug resistant *E. coli* strains was observed on uroepithelial cell. The anti-adherence bioactivity of the proanthocyanidin was detected at concentrations of 10-50 µg/ml with significant bacteriuria. It is probable that proanthocyanidin through A-type linkages either combines to P-fimbriae of bacterial cells or modifies the structural entity of P-fimbriae and inhibits bacterial adherence to uroepithelial cells. The proanthocyanidin exhibited anti-adherence property with multi-drug resistant strains of uropathogenic P-fimbriated *E. coli* with *in vitro* study. It was concluded that the proanthocyanidin may be considered as an inhibitory agent for multi-drug resistant strains of *E. coli* adherence to uroepithelial cells (Gupta *et al.*, 2012).

In a later study cranberry juice and fractions (cranberry juice extract, three fractions containing flavonoid classes including proanthocyanidins, anthocyanins and flavonols, selected sub-fractions, quercetin-3-O-galactosid) were tested for their effect on the surface adhesion of the pathogenic clinical bacterial strain *E. coli* B78 and non-pathogenic, non fimbriated control *E. coli* HB101 with AFM. Adhesion forces of HB101 are small (average force 0.19 nN) and do not change with cranberry treatments, whereas the adhesion forces of the *E. coli* strain B78 (average force of 0.42 nN) showed a significant decrease when treated with cranberry juice extract or fractions (e.g. average force of 0.31 nN with CCE). In particular, the fractions that contained flavonols in addition to PACs were more efficient at lowering the force of adhesion (average force of 0.31 nN-0.18 nN between different sub-fractions containing flavonols and PACs). The sub-fractions containing flavonol glycosides (from juice, fruit and commercial quercetin) all resulted in reduced adhesion of the pathogenic bacteria to the model probe. This suggests the anti adhesive role of other classes of cranberry compounds in conjunction with already known PACs and may have implications for development of alternative anti bacterial treatments (Gupta *et al.*, 2016).

Cranberry phenolic compounds and their potential microbial-derived were tested for their capacity to inhibit the adherence of uropathogenic *Escherichia coli* to T24 epithelial bladder cells. Catechol, benzoic acid, vanillic acid, phenylacetic acid and 3,4-dihydroxyphenylacetic acid showed anti-adhesive activity against UPEC in a concentration-dependent manner from 100-500 μM, whereas procyanidin A2, widely reported as an inhibitor of UPEC adherence on uroepithelium, was only statistically significant ($p<0.05$) at 500 μM (51.3% inhibition). The cranberry extract showed no activity at all (de Llano *et al.,* 2015).

Cranberry xyloglucan-rich fractions inhibited the adhesion of *E. coli* type 1 and P-fimbriated strains to T24 human bladder epithelial cells and that of *E. coli* O157:H7 to HT29 human colonic epithelial cells. Specific binding of p-fimbriated *E. coli* to uroepithelial cells decreased with increasing concentrations (0−5 mg/ml) of oligosaccharide enriched extract in a dose-dependent manner. The oligosaccharide enriched extract showed 6.8 times lower binding affinity to the p-fimbriated *E. coli* compared to the cranberry polyphenolic enriched standard (half-maximal inhibitory concentration 0.82 mg/ml and 0.12 mg/ml, respectively). SSGG xyloglucan oligosaccharides represent a new cranberry bioactive component with *E. coli* anti-adhesion activity and high affinity for type 1 (Hotchkiss *et al.,* 2015).

A2-linked proanthocyanidins reduced the adhesion (up to 75% at the concentration of 50 μg/mL) of strains of *P. mirabilis* and UPEC, as well as the motility and urease activity of the latter. The lower concentrations had a variable efficacy in reducing adhesion of the bacteria in the study, as well as providing discrepant results (Nicolosi *et al.,* 2014).

Anti-biofilm properties

In vitro

Escherichia coli strain HB101pDC1 and non-fimbriated strain HB101 were grown in 10 wt% cranberry juice cocktail (CJC) or 120 μg/mL PACs for 12 consecutive cultures. Biofilm formation was investigated by incubating bacteria in 96- well polyvinyl chloride (PVC) plates and studying the optical density of the solution using the crystal violet method. Both P-fimbriated E. coli HB101pDC1 and the non-fimbriated strain HB101 formed biofilms. Cranberry juice inhibited biofilm formation after the first culture; however, for bacteria grown in PACs, a decrease in biofilm formation was observed with increasing number of cultures. The inhibitory effect was reversible. These results demonstrate that CJC is more effective than isolated PACs at preventing biofilm formation, possibly suggesting that other cranberry compounds also play a role in anti-biofilm activity (Pinzón-Arango *et al.,* 2011).

A concentrated cranberry extract was examined, with a total phenolic content and total anthocyanins content of 90.42 ± 1.80 mg gallic acid/g of DW (DW=dry weight) and 28.95 ± 0.30 mg cyanidin-3-gluco- side/g of DW, respectively against two *Escherichia coli* strains isolated from urine of patients

with pyelonephritis. The cranberry extract limited the ability of bacteria to form biofilm (Wojnicz *et al.,* 2012).

In a later study, the same product was investigated. Results showed that the cranberry extract significantly reduced the growth, enzymatic activities of *E. faecalis* strains isolated from urine and limited biofilm formation. The MICs of cranberry extract against all *E. faecalis* isolates tested were 4.0 mg/mL. The most effective anti-biofilm activity was stated after 72 h of incubation (Wojnicz *et al.,* 2016).

A phenolic-free carbohydrate fraction (cranf1b-F2) was purified from cranberry fruit, comprising predominantly oligosaccharides possessing various degrees of polymerisation. In antimicrobial assays, cranf1b-F2 (at 1.25 mg/ml concentration) reduced biofilm production by the uropathogenic *Escherichia coli* CFT073 strain by over 50% but did not inhibit bacterial growth. Cranf1b-F2 (ranging from 0.625 - 10 mg/ml) also inhibited biofilm formation of the non-pathogenic *E. coli* MG1655 strain up to 60% in a concentration-dependent manner. According to the authors these results suggest that cranberry oligosaccharides, in addition to its phenolic constituents, may play a role in its preventive effects against urinary tract infections (Sun *et al.,* 2015).

A total of 25 fractions from a cranberry extract were isolated using semipreparative HPLC and characterized. Then, the effect on *E. coli* surface hydrophobicity and biofilm formation of the cranberry extract as well as the purest fractions (a total of 13) was tested. The whole cranberry extract presented a powerful antibacterial activity against UPEC while the selected fractions presented a different behavior. Myricetin and quercitrin significantly decreased ($p<0.05$) *E. coli* biofilm formation compared with the control, while dihydroferulic acid glucuronide, procyanidin A dimer, quercetin glucoside, myricetin and prodelphinidin B led to a significant decrease of the surface hydrophobicity compared with the control (Rodríguez-Pérez *et al.,* 2016).

Influence on flagellation and motility

In vitro

Cranberries/PAC

Concentrated cranberry extract with a total phenolic content and total anthocyanins content of 90.42 ± 1.80 mg gallic acid/g of DW (DW= Dry weight) and 28.95 ± 0.30 mg cyanidin-3-gluco- side/g of DW, was examined against two *Escherichia coli* strains isolated from urine of patients with pyelonephritis. The cranberry extract decreased the hydrophobicity of one of the studied *E. coli* strains, reduced swimming motility and adhesion to epithelial cells of both studied strains. Expression of curli was not affected by cranberry extract, the assessment of P fimbriae expression was not reliable due to extract-induced agglutination of erythrocytes. Cranberry extract caused filamentation in both studied *E. coli* strains (Wojnicz *et al.,* 2012).

When UPEC strain CFT073 was grown or exposed to dehydrated, crushed cranberries or to purified cranberry-derived proanthocyanidins (cPACs), expression of the flagellin gene (fliC) was inhibited. In agreement with these results, bacteria grown in the presence of cranberry materials revealed fewer flagella than those in bacteria grown under control conditions. Furthermore, swimming and swarming motilities were hindered when bacteria were grown in the presence of the cranberry compounds (Hidalgo *et al.*, 2011).

In vitro test was performed by Hasegawa *et al* (2017) against twitching motility of uropathogenic Escherichia coli (UPEC) and the antiadhesive properties to human bladder epithelial cells (HTB-9). The E.coli strain K-12 and the strains BK1, BK2, and BK3 were used in this experiment. Proanthocyanidins (PACs) were added at a concentration of 500μM. PACs significantly inhibited the attachment of the

UPEC strains to HTB-9 cells ($p<0.05$), but it did not influence the attachment of K-12 strain to HTB-9 cells.

Compound groups

Cranberry PACs reduced swarming motility with the addition of 100 µg/mL PACs and significantly disrupted the biofilm formation of *P. aeruginosa in vitro* at concentrations as low as 1 µg/mL (40.9%, $p<0.05$). Proteomics analysis revealed significantly different proteins expressed following PAC treatment. The PACs did not kill *P. aeruginosa* at any concentration tested *in vitro*, and thus are not directly antimicrobial.

Cranberry PAC concentrations of ≥16 mg/l significantly reduced biofilm formation in all *C. albicans* strains tested. Further, cranberry PACs were additive in combination with traditional antifungals. Cranberry PACs reduced *C. albicans* adherence to both polystyrene and silicone. Supplementation of the medium with iron reduced the efficacy of cranberry PACs against biofilms (Rane *et al.,* 2014).

Antibacterial activity

In vitro

Cranberry concentrate/extract/juice

The inhibition of the microbial growth has been studied by Lin *et al.* (2011) with microarray technology. GeneChip® *Escherichia coli* genome 2.0 arrays were used to gain insight into the molecular mechanisms involved in the impact of cranberry juice on the properties of *E. coli* growth. The inclusion of 10% cranberry juice in bacterial growth media was found to significantly impact the doubling time of *E. coli* (average doubling time 20 min increased to 107 min). PACs (100, 50, or 10 lg/mL) demonstrated similar inhibition of the growth rate. The gene expression results revealed altered expression of genes associated with iron transport and essential metabolic enzymes as well as with adenosine triphosphate (ATP) synthesis and fumarate hydratase in these cultures. The altered expression of genes associated with iron transport was consistent with the strong iron chelating capability of PACs. The iron depletion effect was confirmed by adding exogenous iron to the growth media. This addition partially reversed the inhibitory effect on bacterial growth observed in the presence of cranberry juice/extracts. Hidalgo *et al.* (2011) reported that PACs induce a state of iron limitation in *E. coli*. Observation of the formation of PAC-Fe complexes confirmed that PACs act as iron chelators.

S. aureus was more susceptible to concentrated cranberry juice (55 ° Brix) inhibition than the other tested microorganisms (14.58 mm halo of inhibition). *L. monocytogenes* was the most resistant (9.5 mm) to the inhibitory action of cranberry juice, showing a significant difference from the inhibition of *P. aeruginosa* (12.16 mm), uropathogenic *E. coli* (11.07 mm)*, Salmonella spp*. (12.62 mm), and *S. aureus*. This study also demonstrated that the inhibitory activity of cranberry juice for *E. coli* took place up to a dilution of 1:20 (Magariños *et al.,* 2008).

Bacillus cereus (1.90-2.55 cm inhibition zones) and *Micrococcus luteus* (2.13-2.40 cm inhibition zones) were the most sensitive among 10 Gram-negative and Gram-positive bacteria when tested with 50 µl acidified ethanol extracts from 4 American cranberry varieties and their press cakes. *E. coli* was the least sensitive (1.50-1.80 cm).Three grams of homogenised berries or berry cake (residue after juice pressing) were extracted with 10 mL of acidified ethanol (95% [v/v] food grade ethanol containing 0.1 M HCl) (Viskelis *et al.*, 2009).

Anti-bacterial activity of *Vaccinium macrocarpon* concentrate on urinary tract *E. coli* was investigated, *in vitro*. *Vaccinium macrocarpon* concentrate at different concentrations was prepared in distilled water and put in wells punched in nutrient agar then inoculated with *E. coli* isolates. A total of 35 isolates of

E. coli were identified out of 96 culture positive specimens of urine and found sensitive to *Vaccinium macrocarpon* ($p<0.000$). Results revealed that *Vaccinium macrocarpon* has antibacterial effect against *E. coli*. Furthermore the antibacterial activity of *Vaccinium macrocarpon* has dose response relationship. Acidic nature of *Vaccinium macrocarpon* due to its pH was not contributory towards its antibacterial effect (Bukhari *et al.,* 2015).

Two commercial cranberry extracts were compared that contain proanthocyanins (PACs) at 4% and 20%. The assessment of antimicrobial activity was performed on Gram-positive *Enterococcus faecalis, Staphylococcus* aureus, Gram-negative *Escherichia coli, Pseudomonas constantini, Proteus mirabilis* and yeast *Candida albicans.* Both extracts showed antimicrobial activity (MIC values range 3-100 µg/ml). In cellular experiments the extracts resulted clearly differentiated in their activity, and the activity was influenced by PACs content. Only in DPPH test the free radical scavenging activity seemed to be directly related to proanthocyanidins content (Menghini *et al.,* 2011).

Three proprietary PAC-"standardised" cranberry extracts (8, 210, 55.3 mg/g PAC) inhibited the growth of the Gram-positive bacteria (*Staphylococcus epidermidis, Staphylococcus aureus*, clinical methicillin-resistant *S. aureus* (MRSA) but not the Gram-negative species (*E. coli*) with minimum inhibitory concentrations in the range 0.02-5 mg/ml. The extracts also inhibited biofilm production by the Gram-positive bacteria but did not eradicate their established biofilm (LaPlante *et al.,* 2012).

Isolated compounds/fractions

The effect of anthocyanin- and proanthocyanidin-rich fractions (50, 200, and 500 µg/ml) isolated from cranberry juice was studied for their antibacterial activity against nine bacterial strains. Activity was assessed by the agar diffusion assay. *Staphylococcus aureus* was the only strain to exhibit some susceptibility to four out of 10 anthocyanin-rich fractions tested. A variable susceptibility of *S. aureus*, *Enterococcus faecalis*, and *Micrococcus luteus* to proanthocyanidin- rich fractions was also observed. *Streptococcus mutans* strains as well as *Escherichia coli* and *Pseudomonas aeruginosa* were not susceptible to any of the cranberry juice samples or fractions at the tested concentrations. There was no clear correlation between Gram-positive or Gram-negative bacterial susceptibility to cranberry juice (Leitão *et al.,* 2005).

The antimicrobial properties of the American cranberry were studied against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Lactobacillus rhamnosus* to determine the effects on growth inhibition, membrane permeability, and injury. Cranberry powder was separated into sugars plus organic acids (F1), monomeric phenolics (F2), and anthocyanins plus proanthocyanidins (F3). Fraction 3 was further separated into anthocyanins (F4) and proanthocyanidins (F5). *L. monocytogenes* was the most susceptible to cranberry fraction treatment with the lowest MIC/MBC for each treatment, followed by *E. coli* O157:H7 and *L. rhamnosus. L. rhamnosus* demonstrated the highest membrane permeability followed by *E. coli* O157:H7, and *L. monocytogenes. L. rhamnosus* demonstrated the highest recovery followed by *E. coli* O157:H7, and *L. monocytogenes*. Each cranberry fraction demonstrated membrane hyperpolarization at their native pH, while F2, F3, and F5 demonstrated membrane depolarization at neutral pH (Lacombe *et al.,* 2013).

The potential of PAC in preventing gut colonization by *E. coli* was examined. *E. coli* attached to and invaded enterocytes by hijacking the host cytoskeletal system, and survived within intracellular vacuoles without causing overt pathology. *E. coli* also resisted post phagocytic killing by macrophages. Exposure of *E. coli* to PAC significantly inhibited invasion in a PAC dose dependent manner; higher molecular weight PAC were more effective in inhibiting invasion. Scanning electron microscopy revealed that PAC exposure disrupted surface structures on *E. coli*. PAC also induced agglutination of *E. coli* and increased killing by macrophages (Shanmuganayagam *et al.,* 2013).

Adjuvant to antibacterial activity

PACs potentiated the antibiotic activity of gentamicin in an *in vivo* model of infection of *P. aeruginosa*. Combination therapy of PACs and gentamicin was significantly ($p<0.05$) more effective in reducing *G. mellonella* larvae (waxworms) death as compared to gentamicin treatment or PAC treatment alone. Gentamicin had an MIC of 1.5 µg/mL against this bacterial strain, gentamicin in combination with PACs was 1.3 µg/ml. The average ratio of death over the 72 h time course between gentamicin alone, PAC alone, and gentamicin-PAC com- bination treatment was 3.4:1, suggesting the significant survival benefit of having the combination treatment (Ulrey *et al*., 2014).

Anti-inflammatory activity, effect on immune system

In vitro

Medium molecular mass fraction from cranberry extract was enriched with flavonoids and procyanidin dimers whereas procyanidin oligomers (DP > 4) were the dominant class of polyphenols in the high molecular mass fraction. Pre-incubation of Caco-2/15 cells with these cranberry extracts counteracted lipopolysaccharide-mediated inflammation as evidenced by the decrease in pro-inflammatory cytokines (TNF-α and interleukin-6), cyclo-oxygenase-2 and prostaglandin E2. Cranberry polyphenols fractions limited both nuclear factor κB activation and Nrf2 down-regulation. Consistently, cranberry procyanidins alleviated OxS-dependent mitochondrial dysfunctions as shown by the rise in ATP production and the up-regulation of Bcl-2, as well as the decline of protein expression of cytochrome c and apoptotic-inducing factor. These mitochondrial effects were associated with a significant stimulation of peroxisome-proliferator-activated receptor γ co-activator-1-α, a central inducing factor of mitochondrial biogenesis and transcriptional co-activator of numerous downstream mediators (Denis *et al.*, 2015).

Seeram investigated several berries and cherries and their anthocyanins for their cyclooxygenase inhibitory activity. Anthocyanins from cranberries showed inhibition of COX-I activities. Cranberries also showed inhibitory activity against COX-II (Seeram *et al.,* 2001).

A methanol extract prepared from of dehydrated cranberries did not directly inhibit the growth of *E. coli* strains ATCC 700336 or ATCC 25922 in concentrations up to 256 µg/ml *in vitro*. However, the methanol extract (CR-ME) inhibited the activity of cyclooxygenase-2, with an IC_{50} of 12.8 µg/ml. Moreover, CR-ME also inhibited the NF-kappabeta transcriptional activation in human T lymphocytes with an IC_{50} of 19.4 µg/ml, and significantly ($p<0.01$) inhibited the release of interleukin (IL)-1beta, IL-6, IL-8 and tumor necrosis factor-alpha from *E. coli* lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells *in vitro*, at a concentration of 50 µg/ml. The extract had no effect on inducible nitric oxide synthase activity in the murine macrophage cell line RAW 264.7. The compounds responsible for this activity were identified as ursolic acid and ursolic acid derivatives. It was concluded that these data suggest CR-ME and its constituent chemical compounds target specific pathways involved in *E. coli*-induced inflammation (Huang *et al.*, 2009).

The aim of this study of Bodet *et al.* (2006) was to investigate the effect of non-dialyzable material prepared from cranberry juice concentrate on the pro-inflammatory cytokine response of macrophages induced by lipopolysaccharides (LPS) from *Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum subsp. nucleatum, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*, and *Escherichia coli*. Interleukin-1 beta (IL-1beta), IL-6, IL-8, tumor necrosis factor alpha (TNF-alpha), and Regulated on Activation Normal T-cell Expressed and Secreted (RANTES) production by macrophages treated with the cranberry fraction prior to stimulation by LPS was evaluated by ELISA. The cranberry fraction was a potent inhibitor of the pro-inflammatory cytokine and chemokine responses induced by LPS.

Cranberry proanthocyanidins (PACs)

Another study investigated the effect of chitosomes on the activation of cranberry proanthocyanidins (PAC) in Raw 264.7 macrophages. About 85% of the PAC that was loaded remained in the chitosomes after release studies for 4 hours in phosphate-buffered saline. Increasing the amount of PAC loaded into the chitosomes caused a dose-dependent attenuation of iNOS and COX-2 expression in LPS-stimulated macrophages. A 2% v/v PAC-loaded chitosomes formulation almost completely attenuated the LPS-induced expression of iNOS and COX-2. PAC-loaded chitosomes were more active than PAC alone, suggesting that the macrophage response to LPS occurs after endocytosis of the PAC-loaded chitosomes (Madrigal-Carballo *et al.,* 2009).

Pierre *et al.* (2013, 2014) in two studies examined the effect of PACs on the intestines following elemental enteral nutrition (EEN). Mice were randomized to receive chow, EEN, or EEN + PACs (50/100 mg/kg body weight) for 5 days. Adding PACs to EEN reversed impaired intestinal barrier function following EEN by improving the gut mucous layer and function through increased goblet cell size and number as well as levels of MUC2 and ileal IL-4, IL-13 and sIgA. Addition of PACs to EEN may support gut-associated lymphoidtissue function and maintain intestinal sIgA levels compared with EEN administration alone.

Table 4: Overview of the main non-clinical data/conclusions related to anti-adherence activity

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
cranberry juice cocktail and fresh cranberry juice	oral as drinking fluid	*In vivo/ In vitro* The bacterial counts of infected bladders of mice in each group were compared to the control group.	Jensen et al. 2017	Reduced bacterial counts were found in the bladder (p< 0.01) of mice drinking fresh cranberry juice or juice cocktail.
cranberry juice cocktail	oral as drinking fluid for 14 days	*Ex vivo/in vitro* Bacterial adherence assay with Urine collected from the mice	Sobota 1984	Adherence of *E. coli* to uroepithelial cells was inhibited by approximately 80% (p<0.01)
cranberry juice cocktail (CJC)	oral as drinking fluid for 30 days oral 240 ml equivalent of 83 mg of proanthocyanidin	*Ex vivo/in vitro* Urine from mice was tested for the ability to prevent P-fimbriated *E. coli* anti-adherence activity to uroepithelial cell surfaces urine examples from volunteers	Howel 2001 Howel 2005	CJC exhibited anti-adherence activity anti-adhesion activity continuously increased in a regular progression, peaking at 4–6 h post-consumption and persisting in the urine for at least 8 h, and persisting in the urine for at least 8 h

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
		bacterial anti-adhesion activity		
25 % Juice, 100 % Juice or water	tube fed with 1 ml 3 times per day	*Ex vivo/in vitro* Urine from rats was tested for capability to decrease of *E. coli* in hemagglutination, urothelium adhesion, nematode killing, and biofilm formation	Chen *et al.,* 2013	decrease in urothelium adhesiveness, 16.7% (p=0.0004) 53.9% ($p=5.9\times10^{-4}$) decrease in biofilm formation
cranberry juice	50 µL ~5 × 10^8 bacteria/plate	*In vitro* determine the ability of cranberry to inhibit P-fimbriated E. coli from binding to human erythrocytes as measured by Hemagglutination	Boland 2016	cranberry juice can block P-fimbriae mediated binding of *E. coli* to human cell
cranberry juice cocktail and juice	1:2 , 1:12 1:50 dilution	*In vitro* the effect on the adherence of Escherichia coli expressing surface lectins of defined sugar specificity to yeasts, tissue culture cells, erythrocytes, and mouse peritoneal macrophages.	Zafriri *et al* 1989	dose dependent inhibition of the adherence of urinary isolates expressing type 1 fimbriae and P fimbriae Gal(Gal=Galactose] but had no effect on a diarrheal isolate expressing a CFA/I adhesion (CFA=Colonization factor antigen). The cocktail also inhibited yeast agglutination by purified type 1 fimbriae.
cranberry juice Cocktail (CJC)	5, 10, and 20 wt.%	*in vitro*	Liu *et al.*, 2006	CJC affected bacterial surface polymer and adhesion behaviour

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
	cranberry juice dilution 2.5, 5, and 10 wt% cranberry juice dilution 5, 10, and 27 wt.% cranberry juice dilution	molecular-scale effect with atomic forrce microscopy Atomic force microscopy (AFM) was used to directly measure the nanoscale adhesion forces between P-fimbriated Escherichia coli (E. coli) and human uroepithelial cells exposed to cranberry juice, Thermodynamic approach was used to calculate the Gibbs free energy of adhesion changes ΔG_{adh}) in the P-fimbriated *E. coli* – uroepithelial cell system measuring contact angles with three probe liquids.	Liu at al 2010 Liu at al 2008	after a short exposure period (<3 h). CJC significantly decreases nanoscale adhesion forces between P-fimbriated E. coli and uroepithelial cells. Dose-dependent inhibition of adhesion of P-fimbriated E. coli. Gadh increased from −19.94 to −15.31mJm−2 and the number of attached bacteria per UC decreased from 50.2±22.9 to 13.6±5.7, when comparing 0 and 5 wt.% solutions
cranberry juice (Ocean spray)	25% juice	E. coli strains JR1 and DS17 were plated on colonizing factor antigen (CFA) agar medium. Transmission electron micrographs were performed on positive control and test bacteria.	Ahuja *et al* 1998	Cranberry juice irreversibly inhibited P-fimbriae. Electron micrographic evidence suggests that cranberry juice acts on the cell wall preventing proper attachment of the fimbrial subunits or as a genetic control preventing the expression of normal fimbrial subunits or both

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
Juice cocktail	27 % by weight	Bacteria were grown in tryptic soy broth supplemented with either light cranberry juice cocktail (L-CJC) or cranberry proanthocyanidins (PACs)	Pinzón-Arango *et al.,* 2009	decrease in adhesion forces
other preparations				
Cranberry powder				
Cranberry powder	3 mg/ml (9 mg proanthocyanidin per g)	*In vitro* cultured bladder epithelial cells and vaginal epithelial cells sytem before and after exposure	Gupta 2007	Cranberry powder decreased mean adherence of E. coli IA2 to vaginal epithelial cells from 18.6 to 1.8 bacteria per cell (p <0.001 Mean adherence of *E. coli* to cultured bladder epithelial cells was decreased by exposure to 50 µg/ml proanthocyanidin extract from 6.9 to 1.6 bacteria per cell (p<0.001)
cranberry powder	5g/kg/day oral for 3-4 days	*in vitro*/ex vivo adults femail pig collected urine tested antiadhession activity using a human red blood cell (A+) anti-hemagglutination assay with	Coleman 2019	PACs were not the target compounds in the bioactive fractions, complex carbohydrates, specifically oligosaccharides, were identified.

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
		uropathogenic P-fimbriated *E. coli*		
Cranberry extracts				
cranberry extracts	PAC equivalent concentrations of 0–180 µg/ml	*in vitro* *Escherichia coli* adhesion to urepithelial cells	Ermel 2012	Around 80% of inhibition of adhesion with the cranberry extracts at equivalent PAC concentration of 50 µg/ml.
Cranberry extract	containing 118 mg of PACs per dose oral 5, 25 and 75 PACs mg/ml,	ex vivo urine was collected after 16 hours and exposed to *Escherichia coli* *in vitro* bladder epithelial cells exposed to *Escherichia coli* to	Risco *et al.,* 2010	oral suspension and tablets 118 mg PACs/animal showed inhibition 83% and 52%respectively 59 mg PACs/animal showed inhibition of *E. coli* adherence (29% after tablets intake and 40% for powder). *In vitro*, the extract showed inhibition of bacterial adherence in all tested concentrations 25%, 36% and 34% respectively
cranberry extract (DER and extraction solvent unknown)	orally for 6 months 1 g for dogs < 25 kg and 2 g for dogs ≥ 25 kg orally for 60 day	*in vivo* 12 dogs with history of recurrent UTI *in vitro* /ex vivo bacterostasis assay with collected urine	Chou 2016	None of the dogs developed UTI bacterial adherence reduced in urine samples obtained at 30 and 60 days, compared with urine samples obtained before extract administration

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
Cranberry extract	100 mg/kg of extract, containing 15% of total PACs 1. oral for 35 days 2. oral single dose	ex vivo antiadhesive activity against E. Coli of urine collected at days 0, 7, 14, 21, and 35 urine collected at 2, 4, 8, and 24 h	Peron *et al.,* 2017	The highest activity showed the 8 h samples. Antiadhesive activity is ascribable to PAC-A metabolites rather than to a direct PAC-A effect
Cranberry extract (V.m) cranberry extract PAC deleted (V.m. extract≠PAC)	proanthocyanidin content = 21% concentration range of 25−200 µg/mL.	*in vitro* laser scanning microscopy and scanning electron microscopy biofilm assay with E. coli and V.m. extract and V.m. extract≠PAC	Rafsanjany 2015	The biofilm mass produced by E. coli 2980 after incubation with V.m. extract was slightly, but not significantly, increased. V.m. extract≠PAC had no influence on biofilm formation of strain 2980. Proanthocyanidin-free cranberry extracts exert antiadhesive activity by interaction with mannose-sensitive type 1 fimbriae of *E. Coli*
Cranberry extract (DER: 25:1)proanthocyanidins (HPLC) > 2.7 %, p.o., 7 days, 900 mg/day	16 volunteers for 7 days 900 mg/day	ex vivo urine samples antiadhesive effect agains uropathogen E. Coli (UPEC)	Scharf 2018	Results indicated inhibition of adhesion of UPEC strain UTI89 to human T24 bladder cells. B-ring substituted flavones and flavonols contribute to the antiadhesive activity against UPEC by inhibition of the FimH-mediated interaction

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
				with the host cell bladder epithelium.
single substances				
A-type proanthocyanidin	75 µg/ml	Measuring the ability to prevent agglutination of both isolated P-receptor resin-coated beads and human erythrocytes.	Foo 200a and 2000b	Inhibition of adherence of uropathogenic isolates of P-fimbriated Escherichia coli bacteria to cellular surfaces containing a-Gal(1 4 4)b-Gal receptor sequences similar to those on epithelial cells in the urinary tract.
cranberry proanthocyanidin	50 mg/300 ml water 500 mg/300 ml water oral as drinking source for 30 days 60 µg/ml	mice urine was tested ability to prevent P-fimbriated *E. coli* anti-adherence activity to uroepithelial cell surfaces HRBC hemagglutination assay,	Howell 2001 Howell 2005	proanthocyanidin treatments exhibited positive anti-adherence activity at certain times during the test period. proanthocyanidins with A-type linkages exhibited *in vitro* bacterial antiadhesion activity
purified proanthocyanidin from fresh cranberry purified proanthocyanidin from dried Cranberry	increasing concentration 25 µg, 50 µg 75 µg, 100 µg	cultured bladder epithelial cells and vaginal epithelial cells system before and after exposure. The anti-adhesion bioactivity was tested by measuring the ability to suppress agglutination of human RBCs (A1, Rh+).	Gupta 2007 Gupta 2012	Mean adherence of E. coli to primary cultured bladder epithelial cells was decreased by exposure to 50 µg/ml proanthocyanidin extract from 6.9 to 1.6 bacteria per cell ($p < 0.001$). Inhibition of adherence of E. coli by proanthocyanidin extract

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model** ***In vivo/ In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
	150 µg/50 µl to 750 µg/50 µl 20 to 100 µ/ml	Bacterial adherence assays on uroepithelial cells and vaginal epithelial cells (VECs)		occurred in linear, dose dependent fashion over a proanthocyanidin concentration range of 75 to 5 µg/ml. The inhibitory effect of proanthocyanidin on hemagglutination by P-fimbriated bacteria, was dependent on the quantity of the proanthocyanidin.Inhibition of adherence to an extent of about 70% with multi-drug resistant E. coli strains was observed on uroepithelial cell. The anti-adherence bioactivity of the proanthocyanidin was detected at concentrations of 10–50 µg/ml.
Cranberry proanthocyanid	345.8 µg/mL	*E. coli* were grown in tryptic soy broth supplemented with cranberry proanthocyanidins (PACs)	Pinzón-Arango 2009	decrease in adhesion forces
Flavonol fraction+PACs	30 % flavonol hexosides	Atomic force microscopy (AFM) was used to quantify the adhesion forces on a clinically isolated bacterial strain	Gupta 2016	flavonol glycosides resulted in reduced adhesion of the pathogenic bacteria
Phenolic compounds	100 µM, 250 µM, 500 µM	Cell culture methodologies anti-	de Liano 2015	1,2 dihydroxybenzene (catechol/pyrocatechol) was found to have a significant and

Herbal preparation tested	Strength Dosage Route of administration	Experimental model *In vivo/ In vitro*	Reference Year of publication	Main non-clinical conclusions
		adhesive capacity on UPEC		concentration-dependent inhibitory effect against UPEC at all assayed concentrations. The same was observed for other benzoic acids which all exhibited inhibition percentages ≥29% at 500 μM
xyloglucan-rich fractions	0 – 5 mg/mL	E. coli CFT073 and UTI89 strains to T24 11 human bladder epithelial cells and E. coli O157:H7 to HT29 human colonic epithelial cells	Hotchkiss 2015	The oligosaccharide enriched extract showed 6.8 times lower binding affinity to the p-fimbriated E. coli compared to the cranberry polyphenolic enriched standard. The half-maximal inhibitory concentration was 0.82 mg/mL

3.1.2. Secondary pharmacodynamics

Anti-proferative activity

Two commercial cranberry extracts were compared that contain proanthocyanins (PACs) at 4% and 20% for antimicrobial, antiproliferative, antiradical and protective properties against oxidative stress on cell lines. Extract at 20% PACs showed higher antiproliferative activity against HepG2 and MCF7 cells, but not against C2C12 cells. Both extracts showed a dose-dependent free-radical scavenging capacity, and a protective effect on the cell damage was also revealed by reduction of intracellular active oxygen species release. Cranberry extracts confirmed antioxidative properties and efficacy in reduction of cell viability that resulted stronger against tumor cells. The pretreatment with cranberry extracts, furthermore, reveal an increase of cell resistance against oxidative stress. In cellular experiments the extracts resulted clearly differentiated in their activity, and the activity was strongly influenced by PACs content. Only in DPPH test the free radical scavenging activity seemed to be directly related to proanthocyanidins content (Menghini *et al*., 2011).

3.1.3. Safety pharmacology

No data available

3.1.4. Secondary pharmacodynamics

No data available.

3.1.5. Conclusions

No data available.

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

Pharmacokinetic interactions

Rats were treated with a „standardised" extract (DER and extraction solvent unknown) obtained from *Vaccinium macrocarpon* in two dosage schemes (14 days, 0.5 mg of proanthocyanidins/kg/day; 1 day, 1.5 mg of proanthocyanidins/kg/day). The aim of this study was to evaluate the effect of anthocyanins and proanthocyanidins contained in this extract on the activity and expression of intestinal and hepatic biotransformation enzymes: cytochrome P450 (CYP1A1, CYP1A2, CYP2B and CYP3A), carbonyl reductase 1 (CBR1), glutathione-S-transferase (GST) and UDP-glucuronosyl transferase (UGT). Administration of cranberry extract led to moderate increases in the activities of hepatic CYP3A (by 34%), CYP1A1 (by 38%), UGT (by 40%), CBR1 (by 17%) and GST (by 13%), while activities of these enzymes in the small intestine were unchanged. No changes in the relative amounts of these proteins were found. The authors concluded that the interactions of cranberry extract with simultaneously administered drugs seem not likely (Bártíková *et al*., 2014).

Cranberry was screened for the potential to inhibit CYP2C8 activity in human liver microsomes. A volume per dose index (VDI) was calculated to determine the volume in which a dose should be diluted to obtain IC_{50} equivalent concentration. Cranberry had a VDI value >5.0 l per dose unit, suggesting a potential for interaction. Based on the calculated inhibition curves the IC_{50} (mean ± SE) value of cranberry was 24.7 ± 2.7 mg/ml (Albassam *et al.*, 2015).

Activity of human CYP3A4 enzyme was used as a parameter to determine the effect of cranberry supplement from nine manufacturers. The content of a cranberry product, equivalent to one capsule, was extracted with methanol. Aliquots (5 ul) of the extract were tested for their ability to inhibit the metabolism of the human CYP3A4 substrate quinine, using an *in vitro* liver microsomal technique. Of nine cranberry products tested, eight products had little or no effect but only one brand caused very strong inhibition (67.2 %) of CYP3A4. The reason for this inhibition is unknown. The effect of cranberry was varied and ranged from 4.4 % activation to 67.2 % inhibition. Lack of effect on human CYP3A4 activity suggests that use of cranberry dietary supplement is unlikely to cause significant interactions with drugs metabolized by CYP3A4 (Wanwimolruk *et al.,* 2012).

Ueswa and Mohri (2006) suggested that cranberry juice has the potential to inhibit the CYP3A mediated activity which was involved in the oxidation of nifedipine (NFP) in both rat intestine microsomes and human liver microsomes. Cranberry juice (CJ) was a potent inhibitor of human and rat CYP3A. Preincubation with 10% vol/vol of CJ and 1 mM NADPH for 10 min resulted in significant inhibition of the NFP oxidation activity of human and rat CYP3A (18.2 and 12.6% decreases, respectively, compared with preincubation experiments without NADPH). In addition, the pharmacokinetic interaction between CJ and NFP *in vivo* was confirmed in rats. In comparison with a control group, the area under the concentration-time curve (AUC) of NFP was approximately 1.6-fold higher when CJ (2 mL) was injected intraduodenally 30 min before the intraduodenal administration of NFP (30 mg/kg). However, the mean residence time, the volume of distribution and the elimination

rate constant were not changed significantly. According to the authors, these data suggest that CJ component(s) inhibit the function of enteric CYP3A.

An *in vivo* study in rats showed a cranberry juice product to inhibit the intestinal first-pass metabolism of the CYP3A substrate nifedipine. However, a clinical study involving the CYP3A probe substrate midazolam and a different cranberry juice product showed no interaction. First, the effects of five cranberry juices, were evaluated on midazolam 1'-hydroxylation activity in human intestinal microsomes. Four of the five brands provided a concentration dependent inhibition of CYP3A activity in the human liver microsomes. However, the extent of inhibition at comparable time point varied between the four brands of cranberry juice. In only 2 out of 5 brands of cranberry juice tested at the higher concentrations (0.5%, v/v) was CYP3A4 activity completely inhibited, which was comparable to the complete inhibition produced by ketoconazole (2 µM concentration) used as a positive control in this experiment (18).One brand of cranberry juice (Juice E) that produced maximum CYP3A inhibition (ablating activity at 0.5% juice (v/v) relative to control) was selected for further *in vivo* characterization. Then, juice E was fractionated to generate hexane-, chloroform-, butanol-, and aqueous-soluble fractions. The hexane- and chloroform-soluble fractions at 50 µg/ml were the most potent, inhibiting by 77 and 63%, respectively, suggesting that the CYP3A inhibitors reside largely in these more lipophilic fractions. Finally, juice E was evaluated on the oral pharmacokinetics of midazolam in 16 healthy volunteers (please see section 5.5.4), which supported the *in vitro* observation (Ngo *et al.,* 2009).

Five types of cranberry juices were compared with those of water on CYP2C9 activity (S-warfarin 7-hydroxylation) in human liver microsomes (HLM). Only one juice inhibited S-warfarin 7-hydroxylation in HLM in a concentration-dependent manner ($p<0.05$), from 20% to >95% at 0.05% to 0.5% juice (v/v), respectively. However, it had no effect on warfarin clearance in healthy participants (please see section 5.5.4) (Ngo *et al.,* 2010).

Ushijima *et al.* (2009) evaluated the ability of cranberry juice to inhibit the human liver microsomal CYP2C9 activity using diclofenac as the probe substrate. The well-established potent CYP2C9 inhibitor sulfaphenazole was used as a positive control. Upon incubation with various amounts of cranberry juice, CYP2C9 activity in the human liver microsomes was inhibited in a concentration dependent manner, such that an IC50 value of 1.14% v/v was established for the cranberry juice. The relative IC50 value established for sulfaphenazole in the same human liver microsomes was 0.4 µM. This confirmed that cranberry juice at a higher dose was a potent inhibitor of CYP2C9, with complete abolition of the metabolism of diclofenac. Greenblatt *et al.* (2006) investigated the ability of cranberry juice to inhibit CYP2C9 mediated hydroxylation of flurbiprofen in a freshly prepared human microsomal system. Cranberry juice showed a concentration dependent inhibition of liver microsomal CYP2C9 and the IC_{50} value for the CYP2C9 inhibition was about 2.5% v/v. Fluconazole inhibited the CYP2C9 mediated activity of human liver microsomes with an IC_{50} value of 29.5 µM (21).

Langhammer & Nilsen (2014) reported that cranberry did not inhibit *in vitro* CYP activities significantly; aqueous or ethanolic extracts of a commercially available cranberry product had IC_{50} over 1000 µg/ml for the investigated enzymes (CYP2D6, 3A4 and 1A2). Incubations were performed with recombinant cDNA-expressed human CYP enzymes in the presence of positive inhibitory controls.

Three triterpenes (maslinic acid, corosolic acid, and ursolic acid) were isolated from *V. macrocarpon*. Their inhibitory potency (IC_{50}) acid was 7.4, 8.8, and < 10 µM, respectively, using HIM as the enzyme source and 2.8, 4.3, and < 10 µM, respectively, using recombinant CYP3A4 as the enzyme source. These *in vitro* inhibitory potencies, which are within the range of those reported for two CYP3A inhibitory components in grapefruit juice, suggest that these triterpenes may have contributed to the midazolam-cranberry juice interaction observed in a clinical study (Kim *et al.,* 2011).

The effect of an ethanolic extract of cranberry pressed juice on UDP-glucuronosyltransferase (UGT) 1A4, UGT1A6, and UGT1A9 activities in human liver microsomes was investigated. A weak inhibition was observed in the case of UGT1A9 with IC_{50} value 260.5 µg/ml. UGT1A9 catalyzes glucuronidation of a wide range of substrates including MPA, propofol, raloxifene, and flavopiridol (Mohamed & Frye, 2011). Choi *et al.* (2014) reported also that cranberry (commercial product, fruit) weakly inhibited UGT1A9 activity (IC_{50}=458±49.7 µg/mL) *in vitro*, but not UGT1A1, UGT1A4, UGT1A6 and UGT2B7.

Absorption

The study of Ou *et al.* (2012) examined the transport of A-type cranberry procyanidin dimers, trimers, and tetramers on differentiated human intestinal epithelial Caco-2 cell monolayers. Procyanidins were extracted from cranberries and purified using chromatographic methods. Fraction I contained predominantly A-type procyanidin dimer A2 [epicatechin-(2-O-7, 4-8)-epicatechin]. Fraction II contained primarily A-type trimers and tetramers, with B-type trimers, A-type pentamers, and A-type hexamers being minor components. Fraction I or II in solution was added onto the apical side of the Caco-2 cell membranes. Data indicated that procyanidin dimer A2 in fraction I and A-type trimers and tetramers in fraction II traversed across Caco-2 cell monolayers with transport ratio of 0.6%, 0.4%, and 0.2%, respectively. This study demonstrated that A-type dimers, trimers, and tetramers were transported across Caco-2 cells at low rates, suggesting that they could be absorbed by humans after cranberry consumption (Ou *et al.,* 2012).

Elimination

The glycosides of flavonoid, anthocyanins and A type proanthocyanidins in cranberry concentrate were characterized and „standardised" using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Cranberry concentrate (1 g/body weight) was orally gavaged to Fischer-344 rats (n=6). Isorhamnetin, myricetin, kaempferol, and proanthocyanidin dimer A2, together with thirteen conjugated metabolites of quercetin and methylquercetin and intact peonidin 3-O-galactoside and cyanidin 3-O-galactoside were identified in the rat urine after cranberry treatment. Very low levels of isorhamnetin (0.48 ± 0.09ng/mL) and proanthocyanidin dimer A2 (0.541 ± 0.10 ng/mL) were found in plasma samples after 1 h of cranberry administration. Although no quercetin was detected in plasma, MRM analysis of the methanolic extract of urinary bladder showed that chronic administration of cranberry concentrate to rats resulted in accumulation of quercetin and isorhamnetin in the bladder. These results demonstrate that cranberry components undergo rapid metabolism and elimination into the urine of rats (Rajbhandari *et al.,* 2011).

Khanal *et al.* (2010) investigated the effects of feeding a commercially available concentrated cranberry powder (CCP) at three different levels, 3.3, 6.6, and 33 g/kg of diet, and the effect of feeding freeze-dried whole cranberry (CB) powder at 50 g/kg of diet. (Epi)catechins were excreted as free and conjugated in both intact and methylated forms. Excretion of conjugated (epi)catechins was as high as 60% of the total consumed in some cases. Excretion of epicatechins, including their methylated forms, ranged from 30 to 47% of the ingested amount, whereas that of catechins, including their methylated forms, ranged from 9 to 31%. Urinary excretion of (epi)catechins was dose dependent and increased with the amount of (epi)catechins present in the diet. In an other study Khanal *et al*. (2014) reported Urinary excretion of 18 Phenolic acids (PA) and their conjugates was, in rats fed AIN93G-based diets containing 5% (dry weight basis) of cranberry (CB). Hippuric, 4-hydroxyphenylacetic, 3-methoxy-4-hydroxyphenylacetic, and 4-hydroxybenzoic acids were excreted in greatest quantity in the urine over a 24 h. Primary phenolic acid excreted in the berry diets was 4-hydroxycinnamic acid for CB. PA were present in conjugated form with cinnamic acid derivatives being 50-70% and phenylacetic acid derivatives conjugated <10%. Conjugated, and not just the free, PA are significant contributors to total urinary excretion.

3.2.1. Conclusions

The EMA Committee for Medicinal Products for human use (CHMP) has evaluated the principal mode of action of proanthocyanidins intended to be used for prevention and treatment of urinary tract infections (EMA/427414/2016). CHMP concluded that metabolites of PACs and other constituents of cranberry exhibit most probably a pharmacological activity. *In vitro* data have shown that cranberry PAC/A-PAC inhibits primarily P-fimbriated uropathogenic strains of E. coli from adhering to uroepithelial cells. The reduction in adhesion forces may be due to changes in bacterial morphology and/or genetically based decreases in P-fimbrial expression. In addition, PAC free extracts also exhibited *in vitro* antiadhesive effects. Furthermore, there is some general evidence mostly from *in vitro* data for other potential effects such as ant-inflammatory due to the presence of flavonoids and terpenic constituents which are likely present in products containing extracts.

Cranberry and PACs were also reported to have an effect on the motility and biofilm production of *E. coli*.

These antibacterial effects and the reported anti-inflammatory effects support the traditional use of cranberry juice/preparations in the prevention/treatment of UTI.

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

3.3.1. Single dose toxicity

Madrigal-Santillan *et al*. (2012) reported that after single oral administration of cranberry ethanolic extract (CEE) in doses up to 5,600 mg/kg bw, no mortality appeared among the treated mice.

Using the up and down procedure in female rats per the Organization for Economic Cooperation and Development (OECD) guidelines, Sengupta *et al*. (2011) observed that the acute oral LD_{50} of cranberry powder „standardised" to 1.5% proanthocyanidin in female Sprague Dawley rats is greater than 5 g per kilogram of body weight.

3.3.2. Repeat dose toxicity

No data available

3.3.3. Genotoxicity

Madrigal-Santillan et al. (2012) demonstrated in their investigation that treatment with cranberry ethanolic extract (CEE) significantly prevents the damage induced by benzo[a]pyrene (B[a]P) an in vivo mouse peripheral blood micronucleus assay.

The experimental groups were organized as follows: a negative control group (without treatment), a positive group treated with B[a]P (200 mg/kg), a group administered with 800 mg/kg of CEE, and three groups treated with B[a]P and CEE (200, 400, and 800 mg/kg) respectively. The CEE and B[a]P were administered orally for a week, on a daily basis. During this period the body weight, the feed intake, and the determination of antigenotoxic potential were „standardised". The low and medium dose of CEE showed a similar effect to the B[a]P group (however, any of these doses were statistically significant from other group), whereas the dose of 800 mg/kg of CEE produced a protective effect at the end of the treatment period. Authors suggest that antioxidant capacity of the extract my be involved in that effect.

Assessor's comment:

This study cannot be considered as regular genotoxicity test and no conclusion can be drawn from it.

3.3.4. Carcinogenicity

No data available

3.3.5. Reproductive and developmental toxicity

Bałan *et al*. (2017) studied the effect of 220 mg of a cranberry extract corresponding to 5500 mg fruit (25:1)) on pregnant and lactating mice by evaluating influence on the morphology and some parameters of spleen and kidney function of their adult progeny. Cranberry extract dissolved in distilled water at a 44 mg/kg b.m. This dose is equivalent to one capsule (human dose) according to the calculation based on body surface area. Six weeks after birth, the morphometry of spleen and kidney, cytometric analysis of spleen lymphocytes, evaluation of humoral response to SRBC (Sheep Red Blood Cells), and examination of serum creatinine/urea concentration, were performed in the offspring. Spleens of progeny from experimental cranberry group differed from the spleens of progeny of control mice in the lower number of lymphatic nodules and their larger diameter. Moreover, more CD19+ and CD8+ lymphocytes than in the control group were found in spleens of animals from experimental group. In the kidneys of the experimental group an increase in the diameter of glomeruli was observed in comparison with the control. Creatinine and urea serum level were not changed. However, a higher concentration of VEGF and bFGF in cranberry treated pups sera in comparison to the controls was registered.

3.3.6. Local tolerance

No data available

3.3.7. Other special studies

No data available

3.3.8. Conclusions

Non-clinical information on the safety of cranberry juice is sparse.

Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed on the herbal preparation in the monograph.

As there is no adequate information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

3.4. Overall conclusions on non-clinical data

Non-clinical data on anti-bacterial (anti-adhesive, anti-biofilm, etc.) and anti-inflammatory activity of cranberry juice/juice cocktail/extract/PACs support the traditional use of cranberry for prevention/treatment of UTI.

The characterization of possible active substances from most *in vitro* and *in vivo* studies is insufficient. The distinction between herbal substance/preparation and specific compounds or compound groups as active substance would be important but in many studies not strictly made.

The EMA Committee for Medicinal Products for human use (CHMP) has evaluated the principal mode of action of proanthocyanidins intended to be used for prevention and treatment of urinary tract infections (EMA/427414/2016). CHMP concluded that metabolites of PACs and other constituents of cranberry exhibit most probably a pharmacological activity. *In vitro* data have shown that cranberry PAC/A-PAC inhibits primarily P-fimbriated uropathogenic strains of E. coli from adhering to uroepithelial cells. The reduction in adhesion forces may be due to changes in bacterial morphology and/or genetically based decreases in P-fimbrial expression. In addition, PAC free extracts also exhibited *in vitro* antiadhesive effects. Furthermore, there is some general evidence mostly from *in vitro* data for other potential effects such as ant-inflammatory due to the presence of flavonoids and terpenic constituents which are likely present in products containing extracts.

The bioavailability of PACs and A-PACs is not fully known. Oligomeric and polymeric PAC are usually not absorbed or to low extent only (< 10%). There are no reports confirming the presence of PACs in the urine.

In vitro and *in vivo* studies have reported that cranberry preparations have effect on different CYP iso-enzmes. Cranberry juice has the potential to inhibit CYP enzymes (CYP3A and CYP2C9) under *in vitro* conditions, and at higher tested amounts of the juice the extent of inhibition was almost similar to the potent inhibitors of CYP3A (ketoconazole) and CYP2C9 (fluconazole). However, under *in vitro* conditions all anthocyanin principles may be available to have a concerted effort in CYP inhibition, which would be not the case under *in vivo* conditions. Potential risk of drug interaction is further assessed in section 5.5.4.

Non-clinical information on the safety of cranberry is limited.

Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed on the herbal preparation in the monograph.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

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

Cranberry juice

It was once thought that cranberry juice made the urinary tract a more acidic, bacteriostatic environment by increasing the hippuric acid content of the urine (Blatherwhick *et al*., 1914 and 1923; Papas *et al*., 1966; Moen, 1962). While the juice is significantly acidic (pH=2.3), scientific studies have not reinforced this hypothesis, as cranberry only caused temporary if any pH changes lasting 10–15 min in most people and only occasionally did cranberry juice contribute enough hippuric acid to the urine to achieve concentrations which are bacteriostatic at pH 5 (Kahn *et al*., 1967; Bodel *et al*., 1959).

Twenty healthy volunteers, 10 men and 10 women, were in a double-blind, randomized, placebo-controlled, and cross-over study. In addition to normal diet, each volunteer received at dinner a single dose of 750 ml of a total drink composed of:

(1) 250 ml of the placebo and 500 ml of mineral water,

or (2) 750 ml of the placebo,

or (3) 250 ml of the cranberry juice and 500 ml of mineral water,

or (4) 750 ml of the cranberry juice

Cranberry juice was provided by Ocean Spray Cranberries, Inc., USA. Each volunteer took the four regimens successively in a randomly order, with a washout period of at least 6 days between every change in regimen. The first urine of the morning following cranberry or placebo consumption was collected and used to support bacterial growth. Six uropathogenic Escherichia coli strains (all expressing type 1 pili; three positive for the gene marker for P-fimbriae papC and three negative for papC), previously isolated from patients with symptomatic urinary tract infections, were grown in urine samples and tested for their ability to adhere to the T24 bladder cell line *in vitro.* There were no significant differences in the pH or specific gravity between the urine samples collected after cranberry or placebo consumption. A dose dependent significant decrease in bacterial adherence was observed, associated with cranberry consumption. Adherence inhibition was observed independently from the presence of genes encoding type P pili and antibiotic resistance phenotypes (Di Martino *et al.,* 2006).

Fifteen of 22 subjects showed significant antiadherence activity ($p<0.05$) against clinical isolates of E. coli to epithelial cells in the urine 1 to 3 hours after drinking 15 ounces (440 ml) of cranberry cocktail (Sobota, 1984).

Six males and 6 females (18-35 years; body mass index, 19-25 kg/m(2)) consumed placebo, cranberry leaf extract beverage, or low-calorie cranberry juice cocktail (LCJC) once in a randomized, double-blind, placebo-controlled cross-over experimental design trial. The washout period between beverages was 1 week. Blood was collected 0, 2, 4, 8, and 24 hours after beverage consumption for measuring oxidative and inflammatory biomarkers. Urine was collected at 0, 0 to 3, 3 to 6, 6 to 9, 9 to 12, and 24 hours postintervention to assess antibacterial adhesion activity. Consumption of LCJC increased ($p < 0.05$) glutathione concentrations and superoxide dismutase activity compared with placebo. Cranberry leaf extract beverage and LCJC consumption had no effect on the inflammatory biomarkers measured as compared with placebo. At 0 to 3 hours postconsumption, urine from participants who consumed cranberry beverages had higher ($p< 0.05$) *ex vivo* antiadhesion activity against P-fimbriated *Escherichia coli* compared with placebo. An acute dose of cranberry beverages improved biomarkers of antioxidant status and inhibition of bacterial adhesion in urine (Mathison *et al.,* 2014).

Whole cranberries

Uropathogenic *E. coli* isolates were obtained from five women with culture-confirmed urinary tract infections (UTIs). The urine sample was collected 2-5 hours after consumption of approximately 42.5 g of sweetened dried cranberries. *E. coli* isolates were incubated separately in each of the four urine samples collected from the five subjects. Bacteria were harvested from the urine and tested for the ability to prevent adhesion of P-fimbriated *E. coli* bacteria using a mannose-resistant hemagglutination assay with human red blood cells (A1, Rh+). One urine sample demonstrated 50% antiadherence activity, two demonstrated 25% activity, and two did not show any increased activity (Greenberg *et al.,* 2005).

Cranberry extract

This study evaluated the antibacterial efficacy of the consumption of cranberry extract powder (70% ethanol) vs. placebo in the urine of healthy volunteers. A first double-blind, randomised, crossover trial involved eight volunteers who had followed three regimens, with or without cranberry, with a wash-out period of at least 6 days between each regimen. Twelve hours after consumption of cranberry or placebo hard capsules, the first urine of the morning was collected. Different Escherichia coli strains

were cultured in the urine samples. Urinary antibacterial adhesion activity was measured in vitro using the human T24 epithelial cell-line, and in vivo using the Caenorhabditis elegans killing model. With the in-vitro model, 108 mg of cranberry induced a significant reduction in bacterial adherence to T24 cells as compared with placebo ($p<0.001$). A significant dose-dependent decrease in bacterial adherence *in vitro* was noted after the consumption of 108 and 36 mg of cranberry ($p<0.001$). The in-vivo model confirmed that *E. coli* strains had a reduced ability to kill *C. elegans* after growth in the urine of patients who consumed cranberry capsules. Overall, these *in vivo* and *in vitro* studies suggested that consumption of cranberry juice represents an interesting new strategy to prevent recurrent urinary tract infection (Lavigne *et al.,* 2008).

Two separate bioassays (a mannose-resistant hemagglutination assay and an original new human T24 epithelial cell-line assay) have assessed the ex-vivo urinary bacterial anti-adhesion activity on urines samples collected from 32 volunteers in a randomized, double-blind versus placebo study. An *in vivo* Caenorhabditis elegans model was used to evaluate the influence of cranberry regimen on the virulence of *E. coli* strain. The results indicated a significant bacterial anti-adhesion activity in urine samples collected from volunteers that consumed cranberry extract powder (70% ethanol) compared to placebo ($p<0.001$). This inhibition was clearly dose-dependent, prolonged (until 24 h with 72 mg of PAC) and increasing with the amount of PAC equivalents consumed in each cranberry powder regimen. An *in vivo Caenorhabditis elegans* model showed that cranberry acted against bacterial virulence: *E. coli* strain presented a reduced ability to kill worms after a growth in urines samples of patients who took cranberry capsules. This effect is particularly important with the regimen of 72 mg of PAC (Howell *et al.,* 2010).

For investigation of the molecular interaction of cranberry extract with adhesins of uropathogenic Escherichia coli (UPEC), urine from four volunteers consuming „standardised" cranberry extract (proanthocyanidin content=1.24%, 600 mg/day) for 7 days was analysed within *ex vivo* experiments, indicating time-dependent significant inhibition of 40-50% of bacterial adhesion of UPEC strain NU14 to human T24 bladder cells (Rafsanjany *et al.,* 2015).

Three different cranberry extracts were developed containing a „standardised" level of 36 mg of PACs. This randomized, double-blind, placebo controlled, *ex vivo*, crossover, acute study was designed to compare the anti-adhesion activity exhibited by human urine following consumption of three different cranberry extracts on uropathogenic P-fimbriated Escherichia coli in healthy men and women (n=20). *Ex vivo* anti-adhesion activity was measured using participants' urine following cranberry ingredients consumption. Twenty participants (1:1 gender ratio) were tested during placebo and cranberry phases in a crossover design with AAA measured immediately prior to and at 5 time points (6, 9, 12, 24 and 36 h) after consumption of the product. All three cranberry extracts significantly increased anti-adhesion activity in urine. from 6 to 12 hours after intake of a single dose „standardised" to deliver 36 mg of PACs (as measured by the BL-DMAC method), versus placebo (Howell *et al.,* 2015).

Two groups of 12 female volunteers each, aged between 18 and 65 years, were enrolled, one group with negative history and one group with positive history of recurrent cystitis. Subjects were treated with the cranberry extract (36 mg proanthocyanidins) or placebo in a random, cross-over, double-blinded sequence for one week in each of the two treatment sequences. Urine samples were collected at the beginning and the end of each study period. Tests of bacterial adhesiveness were performed with two strains of *E. coli* (ATCC 25922 and ATCC 35218) on HT1376 human bladder carcinoma cells. Significant reductions of bacterial adhesiveness were observed in women who received cranberry extract (-50.9%; p less than 0.0001), regardless of their medical history and the treatment period in the cross-over sequence. No changes were observed with placebo (-0.29%; n.s.) (Tempera *et al.,* 2010).

To evaluate the effect of cranberry extract (PAC-A ~ proanthocyanidin-A) on the *in vitro* bacterial properties of uropathogenic (*E. coli*) and its efficacy/tolerability in patients with subclinical or uncomplicated recurrent UTI (r-UTI). 72 patients with r-UTI were enrolled as per protocol (November 2011 to March 2013) in this prospective study, to randomly receive (PAC-A: group I, 36) or (placebo: group II, 36), for 12 weeks. Any change/reduction in the incidence of r-UTI at 12 weeks was construed to be the primary endpoint of this study. After 12 weeks, bacterial adhesion scoring decreased (0.28)/(2.14) in group I/II ($p<0.001$); 32/36 (88.8 %) and 2/36 (5.5 %) in groups I and II, respectively, turned MRHA negative ($p<0.001$); biofilm ($p<0.01$) and bacterial growth ($p<0.001$) decreased in group I; microscopic pyuria score was 0.36/2.0 in group I/II ($p<0.001$); r-UTI decreased to 33.33 versus 88.89 % in group I/II ($p<0.001$); mean subjective dysuria score was 0.19 versus 1.47 in group I/II ($p<0.001$), while mean urine pH was 5.88 versus 6.30 in group I/II ($p<0.001$). No *in vitro* antibacterial activity of cranberry could be demonstrated, and no adverse events were noted. The overall efficacy and tolerability of „standardised" cranberry extract containing (PAC-A) as a food supplement were superior to placebo in terms of reduced bacterial adhesion; bacterial MRHA negativity; urine pH reduction; and in preventing r-UTI (dysuria, bacteriuria and pyuria). Larger randomized controlled trials are needed to elucidate the precise role, exact dose and optimal duration of PAC-A therapy in patients at risk of r-UTI (Singh *et al.*, 2016).

This study assessed the effect of an 8 week consumption of dried cranberry juice (DCJ) on 65 healthy young women. Basic biochemical and hematological parameters, antioxidant status, presence of metabolites in urine, and urine *ex vivo* ntiadherence activity were determined throughout the trial. A 400 mg amount of DCJ/day had no influence on any parameter tested. A 1200 mg amount of DCJ/day resulted in a statistically significant decrease in serum levels of advanced oxidation protein products. This specific protective effect against oxidative damage of proteins is described here for the first time. Urine samples had an inhibitory effect on the adhesion of uropathogenic Escherichia coli strains, but no increase in urine acidity was noted. Hippuric acid, isomers of salicyluric and dihydroxybenzoic acids, and quercetin glucuronide were identified as the main metabolites (Valentova *et al.,* 2007).

Assessors comment

Preliminary ex vivo/in vitro data suggest that cranberry constituents or metabolites present in human urine inhibit bacterial adhesion to uroepithal cell (antiadhesive activity), which support the reported in non-clinical ex vivo/in vitro antiadhesive effects. The clinical relevance of these findings is currently not known.

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

Urine samples at baseline and post 7 day consumption of cranberry powder (36 mg PAC equivalent/day) were collected from ten human subjects. Supplementation of cranberry powder resulted in statistically significant lowering of eight proteins/peptides in the urine. Many of these proteins seem to play regulatory role in immune processes and tumorigenesis (Krueger, 2013).

The plasma and urine of healthy young men after consumption of a cranberry juice (787 mg (poly)phenols) were examined. A total of 60 cranberry-derived phenolic metabolites were identified, including: sulfates of pyrogallol, valerolactone, benzoic acids, phenylacetic acids, glucuronides of flavonols, as well as sulfates and glucuronides of cinnamic acids. The most abundant plasma metabolites were small phenolic compounds, in particular hippuric acid, catechol-O-sulfate, 2,3-dihydroxybenzoic acid, phenylacetic acid, isoferulic acid, 4-methylcatechol-O-sulfate, α-hydroxyhippuric acid, ferulic acid 4-O-sulfate, benzoic acid, 4-hydroxyphenyl acetic acid, dihydrocaffeic acid 3-O-sulfate, and vanillic acid-4-O-sulfate. Some benzoic acids, cinnamic acids, and flavonol

metabolites appeared in plasma early, at 1-2 h post-consumption. Others such as phenylacetic acids, benzaldehydes, pyrogallols, catechols, hippuric and dihydrocinnamic acid derivatives appear in plasma later (Tmax 4-22 h). The 24 h urinary recovery with respect to the amount of (poly)phenols consumed was 6.2%. PAC monomers catechin and epi- catechin were not detected in plasma or urine. It was not possible to quantify any PAC (Feliciano *et al.,* 2016).

Eleven healthy volunteers consumed 200 ml of cranberry juice containing 650.8 µg total anthocyanins. Urine samples were collected within 24 h before and after consumption. Six of 12 anthocyanins identified in cranberry were „standardised" in human urine. Among these, peonidin 3-O-galactoside, the second most plentiful anthocyanin in the juice, was found most abundantly in urine within 24 h, corresponding to 41.5 nmol (56.1% of total anthocyanins). The urinary levels of anthocyanins reached a maximum between 3 and 6 h after ingestion, and the recovery of total anthocyanins in the urine over 24 h was estimated to be 5.0% of the amount consumed. Proanthocyanidins were not analysed. Milbery *et al* reported, in a study performed on 15 healthy volunteers, that thee total recovery of urinary anthocyanin was 0.79 +/- 0.90% of the dose delivered (urine samples were collected between baseline (0 h) and 4 h after consumption of 480 mL cranberry juice (54% juice; 835 mg total polyphenols; 94.47 mg anthocyanins)). These data are in agreement with the pharmacokinetics of anthocyanins from other foods suggesting that cranberry anthocyanins are poorly absorbed and rapidly removed from plasma (Milbury *et al.*, 2010).

After single ingestion of cranberry syrup Iswaldi *et al.* (2013) identified free coumaroyl hexose (isomer 1 and 2), dihydroxybenzoic acid, caffeoyl glucose, dihydroferulic acid 4-O-β-d-glucuronide, methoxyquercetin 3-O-galactoside, scopoletin, myricetin and quercetin, together with other 23 phase-I and phase-II metabolites, including various isomers in human urine. The allocation of these metabolites to original phenols and polyphenols in the syrup was not possible.

A single-dose pharmacokinetic trial was conducted in 10 healthy adults ⩾50y to evaluate the acute (24-h) absorption and excretion of flavonoids, phenolic acids and proanthocyanidins (PACs) from a low-calorie cranberry juice cocktail (54% juice). Inter-individual variability was observed in the C_{max} and T_{max} of many of these compounds in both plasma and urine. The sum total concentration of phenolics detected in plasma reached a peak of 34.2 µg/ml between 8 and 10h, while in urine this peak was 269.8 µg/mg creatinine, and appeared 2-4h earlier. PAC-A2 dimers could be „standardised" in human urine. The T_{max} of PAC-A2 at 11 h suggests that most of this compound was absorbed in the lower GI tract after being produced from oligomers and polymers via catabolism by gut microbiota. Thus the authors concluded, urinary PAC-A2 is unlikely reflective of the specific bioavailability of PAC-A2 from cranberry intake (McKay *et al.,* 2015).

Five healthy, non-smoking, premenopausal women (20-30 years of age) were assigned to consume a cranberry juice cocktail containing 140 mg proanthocyanin and 35 kilocalories at 237 mL/day, according to a progressive dosing design of 7 weeks. Eleven 24 h and morning spot urine samples each were collected from each subject. A reliable, sensitive method for the detection of proanthocyanin dimer A-2 in urine using liquid chromatography with tandem mass spectrometry was developed with a limit of quantitation of 0.25 ng/mL and a relative standard deviation of 7.26%, precision of 5.7%, and accuracy of 91.7%. While proanthocyanin dimer A-2 was quantifiable in urine, it did not appear to be excreted in a concentration that corresponded to the dosing schedule and intake of cranberry juice (Walsh *et al.,* 2016).

Absorption and excretion of twenty cranberry-derived phenolics were studied following the consumption of 240 ml cranberry concentrate juice, 55 g sauce, and 40 g dried fruits by 11 healthy human volunteers. Significant increases in the sum of plasma phenolics were observed with different concentration peaks (between 0.5 and 2 h) for individual subjects. Some of the phenolics, such as trans-cinnamic, vanillic, p-coumaric acids, and catechin showed second plasma concentration peaks.

All of cranberry-derived phenolics increased significantly in urine samples after the intake of each cranberry product. The high molecular weight quercetin and myricetin, which were abundant in cranberry foodstuffs, were not found in either plasma or urine samples (Wang *et al.,* 2012).

In general proanthocyanidins exhibit low oral bioavailability owing to poor water solubility and extensive presystemic metabolism; they are minimally absorbed due to nonhydrolyzable bonds between monomeric subunits and a propensity to bind proteins through hydrogen bonding. Because of this poor absorption, more than 95% of PACs remain in the intestinal lumen during transit (Upton, 2016). These compounds have very low bioavailability which is further decreased as oligomeric PAC molecular weight increases (Feliciano *et al., +2015)*. Few pharmacokinetics studies have been carried out on cranberry specific PACs due to the structural complexities as well as the lack of commericial standards (Di Martino *et al.,* 2006).

Reviews conclude, that although dimers (procyanidin A2) have been detected in urine, cranberry PAC overall are minimally absorbed because of non-hydrolyzable bonds between monomeric subunits and a propensity to bind proteins by hydrogen bonding (Krueger *et al.,* 2013). The degree of procyanidin polymerization has a major impact on their fate in the body characterized by a poor absorption (95% remain in the intestinal lumen) through the gut barrier and a limited metabolism by the intestinal microflora as compared to catechin (Gonthier *et al.*, 2003).

4.2. Clinical efficacy

There are numerous clinical studies performed with cranberry juice/extracts.

In accordance with the guideline 'Assessment of clinical safety and efficacy in the preparation of EU herbal monographs for well-established and traditional herbal medicinal products' (EMA/HMPC/104613/2005 – Rev. 1), the assessment should also include if the products reported on the EU market by the NCAs in the market overview can be considered as similar to the product studied in relevant clinical studies found in the literature. Therefore, the scope of the assessment is the prevention/treatment of UTI, only studies regarding this condition were included below, followed by an assessment on comparability between the products in the studies and reported products on the EU market.

Furthermore, the 'Guideline on the evaluation of medicinal products indicated for treatment of bacterial infections' (CPMP/EWP/558/95 rev.2) and the 'Addendum to the guideline on the evaluation of medicinal products indicated for treatment of bacterial infections to address indication-specific clinical data requirements' (EMA/CHMP/351889/2013) have been taken into account. For example, there should be a clear definition of "breakthrough" cases in the studies and microbiologically confirmed UTI is considered the highest evidence of a breakthrough.

Considering the definition of uncomplicated UTI, studies in for example the following patient populations have been excluded: men; pregnant women; allografted patients; patients with kidney disorders; patients with catheter associated UTI; patients with multiple sclerosis; patients with spinal cord injuries; and patients with cancer.

Beside these investigations, cranberry preparations were tested for clinical efficacy for instance on lipid, glucose, C-reactive protein levels, blood pressure, perisomal skin conditions, kidney stone formation, vulvovaginal candidiasis, Helicobacter pylori infection, benign prostatic hyperplasia glycaemic response or metabolic syndrome. There is no information available that cranberry products have been in medicinal use for more than 10 years in EU in these indications. Thus, these studies will not be considered for a well-establish use monograph.

4.2.1. Dose response studies

A concentrated cranberry liquid blend (3875 mg (**cranberry concentrate** [4:1], ascorbic acid, D-mannose, fructo-oligosaccharides, and bromelain) per 30 mL), was administered orally at 15, 30, 45, 60, and 75 mL daily for 12 weeks to women (average age 46.5 ± 12.8 years) with a history of 2.78 ± 0.73 rUTIs <6 months. Each group of 6 subjects was assigned to a successive dose group, starting at 15 mL and increasing in each group by 15 mL to a maximal dose target of 90 mL/d. Up to 6 doses of UTI-STAT with Proantinox were designed to be studied. If none of the women in the group had experienced a dose-limiting toxicity after the 4-week visit, the dose was escalated to the next greater level for the 6 subsequent subjects. Blood and urine samples were collected at baseline and weeks 4 and 12. The primary endpoints were the safety, tolerability, and maximal tolerated dose. The secondary endpoints were the efficacy with regard to rUTI and quality-of-life (QOL) symptoms. A total of 28 subjects were included in the study. Of these 28 women, the data from 23 were analyzable. The maximal tolerated dose of UTI-STAT was 75 mL/d, and the recommended dose was set at 60 mL/d. The authors also reported that the secondary endpoints showed that only 2 (9.1%) of 23 women reported a rUTI, a markedly better rate than the historical data. Epidemiologic data have demonstrated that most women with rUTIs have an average of 2-4 episodes annually. At 12 weeks, the reduction in worry about rUTIs and increased QOL with regard to the physical functioning domain and role limitations from physical health domain, as measured by the Medical Outcomes Study short-form 36-item questionnaire, were statistically significant (70.6 ± 14.8 versus 89.6±17.2, p=0.0097, and 85.0±6.4 versus 97.2±5.6, P = 0.028) compared to baseline data in this open study. A lower American Urological Association Symptom Index indicating greater QOL was also statistically significant (from baseline (11.6 ±6.7) to week 4 (5.4 ± 3.3; P =0.39) to week 12 (6.6 ± 5.4; p=0.045). Dose escalation proceeded to 75 mL/d, at which point 3 of 3 patients developed adverse events, including diarrhea, headache, and heartburn at the 75 mL/d dose (Efros *et al.,* 2010).

Assessor's comment:

Limitation of the study that it was not a placebo controlled study, the number of the patient is low. Dose-response relationship was not investigated.

Whole cranberry powder

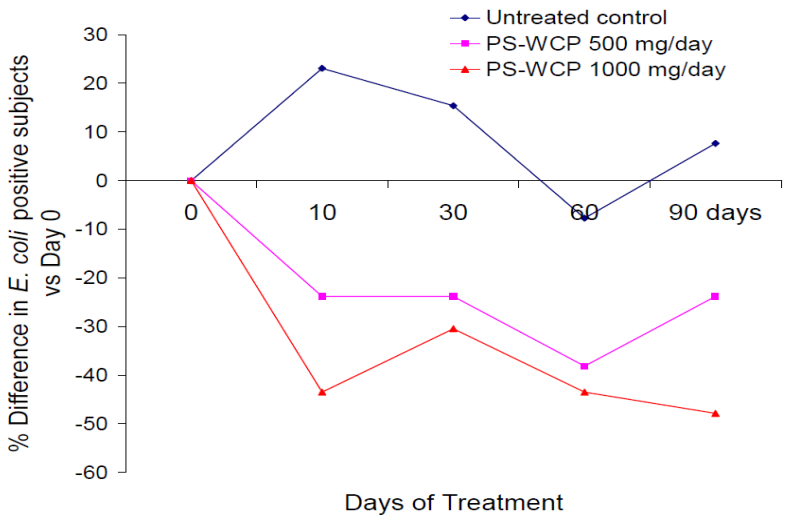
A dose-response study was designed to understand the impact and safety profile of a proanthocyanidin **„standardised" whole cranberry powder** (PS-WCP, „standardised" to 1.5% proanthocyanidins using a proprietary process, equal to 14.4 mg/g PACs,) on reducing the recurrences of symptomatic UTI in culture-positive subjects. A 90-day randomized clinical trial including an untreated control group with a total of 60 female subjects between 18-40 years of age was conducted. Study subjects were randomly selected and assigned to three groups including an untreated control group (n=16), a low dose (250 mg twice daily, n=21) and a high dose (500 mg twice daily, n=23) treatment group. The safety of PS-WCP was assessed by evaluation of biochemical and hematological parameters on days 10, 30, 60 and 90 during the study, comparing the values with those at the baseline. Occurrence of UTI at baseline and during the follow-up period was characterized by the presence of symptoms and Escherichia coli in the culture of urine samples. At the end of the 90-day treatment period, no significant changes were observed in the hematological and serum biochemical parameters. At the end of the study, change in the presence of *E. coli* in the untreated control group was not significant (p=0.7234), whereas, there was significant reduction (p<0.05) in the subjects positive for E. coli in both the high dose (from 74% % to 26.1%) and low dose treatment groups (from 66.7 % to 42.8 %), compared to baseline evaluation (see table 5).

Table 5 Presence of urinary *E. coli* and other uropathogens in different groups at baseline evaluation and at the end of the follow up period of the study. The figures in parentheses represent the percentages, and were calculated from the numbers of subjects in the respective groups.

Group	Baseline (0 day)		90 Day	
	E. coli	*Other uropathogens**	*E. coli*	*Other uropathogens**
Untreated control (n=13)	4 (30.8)	11 (84.6)	5 (38.5)	12 (92.3)
PS-WCP 500 mg/day (n=21)	14 (66.7)	8 (38.1)	9 (42.8)	7 (33.3)
PS-WCP 1000 mg/day (n=23)	17 (74)	10 (43.5)	6 (26.1)	8 (34.8)

* Include *Klebsiella spp, Staphylococcus,* and *Enterobacter spp.*

Figure 1 Percentage difference in *E. coli positive subjects in the control and treatment groups at 10, 30, 60 and 90 day evaluation vs. baseline evaluation.*



Symptomatic relief was also reported in the low and high dose treatment groups, while none was reported by subjects in the untreated control group. The authors conclude, PS-WCP was safe and reduced the number of E. coli positive subjects at both the 500 mg and 1000 mg dose levels (Sengupta *et al.*, 2011).

Assessor's comment:

Clinically relevant reduction (from 74% % to 26.1% p<0.05) was seen in the subjects positive for E. coli in the high dose group (500 mg twice a daily „standardised" whole cranberry powder (PS-WCP, „standardised" to 1.5% proanthocyanidins). However, there was no placebo control in the study and the non-treated control group included a lower number of participants than the treatment groups. In

addition, the presence of Escherichia coli in the culture of urine samples at baseline was lower in the control group than in the two groups receiving cranberry.

The aim of the pilot double-blind, randomized, placebo-controlled trial (Bianco *et al* 2012) was to identify the optimal dose of cranberry capsules that reduced the incidence of bacteriuria plus pyuria over one month. Older nursing home residents were grouped into 3 arms in which they received 3 cranberry capsules daily (108mg proanthocyanidin (PAC)), 2 cranberry plus one placebo capsule daily (72mg PAC), 1 cranberry plus two placebo capsules daily (36mg PAC), and 3 placebo capsules daily for 30 days. The primary outcome was episodes of bacteriuria plus pyuria at 7, 14, 21, and 28 days of cranberry capsule treatment. Participants were stratified by presence or absence of baseline bacteriuria with 20 participants randomized by strata to each arm of the study.

Demographics of the 80 participants included: 98% white(n=78), mean age 89.2 years(S.D., 7 years), and mean number of comorbidities 4.1(S.D., 1.7). Most participants were totally dependent in bathing (54%) and had some bowel(67%) and bladder(76%) incontinence. Of 80 baseline urine cultures, 1 had no growth, 8 had ≤100,000 cfu/ml, 41 had >100,000 cfu/ml, and 30 had mixed flora (3 or more organisms).

Results of bacteriuria plus pyuria by cranberry capsule group are provided in Table 1.

Table 1

Cross-classification of cranberry capsule dose and presence of bacteriuria plus pyuria

Treatment Group	*E.coli* bacteriuria plus pyuria	Other bacteriuria plus pyuria[a]	Not growth[b]	Total
No cranberry capsules	33 (43.4%)	5 (6.6%)	38 (50.0%)	76
One cranberry capsule	29 (40.3%)	4 (5.6%)	39 (54.2%)	72
Two cranberry capsules	23 (29.9%)	10 (13.0%)	44 (57.1%)	77
Three cranberry capsules	25 (34.3%)	12 (16.4%)	36 (49.3%)	73
Total	110	31	157	298[c]

[a] Other bacteriuria plus any WBCs is >100,000 cfu/ml of a pathogen other than *E.coli* plus any WBCs. For no cranberry capsules, 3 were Proteus and 2 were Klebsiella species. For one cranberry capsule, 1 was Proteus and 3 were Klebsiella species. For two cranberry capsules, 2 were Proteus species, 1 Enterococcus, 4 beta-hemolytic Streptococci, 2 viridans Streptococci, and 1 Morganella morganii. For three cranberry capsules, 4 were Klebsiella, 3 Enterococcus, 4 Citrobacter freundii, and 1 coagulase negative Staphylococci.

[b] Not growth includes no growth, growth < 100,000, growth >100,000 but no WBCs, and mixed flora. Of 157 not growth, 19 were no growth, 23 were <100,000, 4 were growth >100,000 but no WBCs, and 111 were mixed flora.

[c] There were 22 missing either urine culture or urinalysis of the 320 total expected samples.

Abbreviation: WBC (white blood cell)

E.coli bacteriuria was reduced, but bacteriuria with other pathogens did not show this same pattern of results Since the effect of two and three capsules was comparable and to reduce capsule burden, further investigation of two cranberry capsules daily in nursing home residents is warranted to determine if the reduction of E.coli bacteriuria is sustained over a longer period of time and whether it impacts clinical outcomes related to UTI (e.g., hospitalization, antibiotic therapy for UTI).

Assessor's comment:

The composition of the cranberry capsules was not provided, only the PAC content (36 mg)/capsule is mentioned. Although the E.coli bacteriuria was reduced, but the difference between the placebo and the treated groups cannot be considered clinically relevant (9.1-13.4 %).

4.2.2. Clinical studies (case studies and clinical trials)

Men and women

Acute urinary tract infection

Papas *et al* (1966) investigated the effects of **cranberry juice** in the treatment of acute infection of the urinary tract. 60 adults patients presented definite symptoms of acute urinary tract infections such as frequency, dysuria, urgency and nocturia. Of the 60 patients, 44 were females, thirty of the women were under 40 years of age; 11 of the males were over 40. 26 of the patients had some prior history of urinary tract infection. All patients were without overt evidence of underlying uropathy or predisposing disease.

After three weeks of treatment with 16 ounces of cranberry juice per day, 53 per cent of the patients had a positive clinical response. Moderate improvement was noted in an additional 20 percent of the patients. Exceptional patient tolerance to cranberry juice was noted. Infection persisted or recurred during the six weeks after treatment in 27 of the patients. Eight of the 27 patients were asymptomatic. negative urine cultures were noted in 17 patients at six week post-therapy.

Prevent urinary tract infection

Gibson *et al* (1991) presented the result of a study in which twenty eight nursing home patients drunk 4 to 6 ounces of **cranberry juice cocktail** (120 ml-180 ml) for 7 weeks appeared to prevent urinary tract infections in 19 of the 28 nursing home patients. Ten patients had no leukocytes or nitrites in their urine as shown by negative test reaction confirmed by microscropic examination. Nine patients had trace to 2+ leukocytes but negative nitrites. These results were confirmed by having an average of 6 or more leukocytes and a small to moderate numbers of bacteria seen in high power microscropic examination.

Women with recurrent UTI

Cranberry/Cranberry juice cocktail (27% juice)/concentrate

Already in 1994 a randomised double blind, placebo controlled trial was performed to determine the effect of regular intake of 300 ml **cranberry juice cocktail** daily (Ocean Spray) on bacteriuria and pyuria in 153 elderly women (Avorn 1994). Bacteriuria with pyuria was found in 28.1% of urine samples in the placebo group and 15.0% in the randomized to the cranberry beverage. The difference was not present in the first month, but appeared most strikingly between month 1 and 2 and remained fairly stable throughout the rest of the trial (6 months). Out of the 473 urine samples collected in the cranberry group 20 (4%) had bacteriuria and pyuria concurrent with the subject's reporting urinary tract symptoms, compared with 37 (7%) of 498 urine samples in the placebo group, however, the result was not statistically significant.

In a retrospective cross-sectional study and a longitudinal cohort study, Dignam *et al* (1998) evaluate the effect of daily intake of **cranberry juice** on the incidence of symptomatic urinary tract infections (UTIs) in long-term care (LTC) 538-bed facility residents, both men and women.

Four ounces of cranberry juice were given to all LTC facility residents during the 8 months intervention period. The residents who did not drink the cranberry juice were subsequently offered 6 Azo-cranberry capsules in divided doses per day.

The cross-sectional study revealed that there were 545 UTIs during the 20-month pre-intervention period (January 1994 to August 1995) with a monthly average of 27.2 (± 4.7, range, 17-34), compared with 164 UTIs during the 8-month intervention period (February 1996 to September 1996) with a monthly average of 20.5 (± 7.7; range, 12-37). There was a significant reduction of symptomatic UTI rates between these two periods, with a t ratio of 2.84 (P = 0.008).

In the longitudinal cohort, there were 113 residents who were present for at least 8 months of the pre-intervention period (January 1995 to August 1995) and the entire 8 months of the intervention period. In this cohort, there were 103 UTIs during the pre-intervention period with a monthly average of 12.8 (± 3.0; range, 7-17), compared with 84 UTIs during the intervention period with a monthly average of 10.3 (± 4.4; range, 7-21). This reduction in symptomatic UTI rates, however, did not reach statistical significance, with a t ratio of 1.31 (P= 0,212).

In this randomized, double-blind, placebo-controlled, multicentre clinical trial, women with a history of a recent UTI, the daily consumption of a cranberry beverage for 24 wk produced a 39% (95% CI: 9%, 59%) reduction in clinical UTI episodes. The volunteers were assigned to consume one 240 ml serving of **cranberry juice cocktail** (27% cranberry juice)/d (n=185) or a placebo (n=188) beverage for 24 weeks. The active study beverage contained filtered water, cranberry juice from concentrate, fructose, natural flavours, pectin, sodium citrate, acesulfame- potassium, and sucralose. The primary outcome was the clinical UTI incidence density, which was defined as the total number of clinical UTI events (including multiple events per subject when applicable) per unit of observation time. The mean age was 40.9 y, and characteristics were similar in both groups. Compliance with study product consumption was 98%, and 86% of subjects completed the treatment period in both groups. There were 39 investigator-diagnosed episodes of clinical UTI in the cranberry group compared with 67 episodes in the placebo group (p=0.016). The rate of clinical UTI with pyuria episodes was also reduced by 37% (95% CI: 3%, 60%, p=0.037) although no difference between the groups was observed for microbiologically positive UTIs (p=0.914).The authors conclude that one clinical UTI event was prevented for every 3.2 woman-years (95% CI: 2.0, 13.1 woman-years) of the cranberry intervention. (Maki *et al*. 2016). This study is concordant with those of Barbosa-Cesnik *et al.* (2011) in showing no significant difference in culture positive UTI incidence.

The aim of the study performed by Maki *et al*. (2016) was to assess relationships between clinical predictors of urinary tract infection (UTI) and effects of cranberry juice consumption on recurrence in a post hoc analysis of a 24-week, randomized, double-blind, placebo-controlled, multicentere clinical trial in women with a recent history of UTI.

Participants consumed a cranberry (n = 185) or placebo (n = 188) beverage (240 mL) daily. Odds ratios (OR) from 20 candidate predictor variables were evaluated in univariate analyses to assess clinical UTI incidence relationships in the placebo group. A multivariate logistic regression model was developed. The effects of cranberry juice consumption were evaluated in subsets categorized by the likelihood of a UTI event based on the prediction model.
In the placebo group, the final multivariate regression model identified four variables associated with the odds for having ≥ 1 UTI: intercourse frequency ≥ 1 time during the prior 4 weeks (OR: 2.36; 95% confidence interval [CI]: 0.98, 5.71; p = 0.057), use of vasectomy or hormonal methods for contraception (OR: 2.58; 95% CI: 1.20, 5.58; p = 0.016), most recent UTI < 90 days prior to screening (OR: 2.28; 95% CI; 1.12, 4.67; p = 0.024), and living in France compared with the United States (OR: 0.17; 95% CI: 0.04, 0.79; p = 0.024). Three propensity categories were investigated (24-week probability < 10%, 10%-21%, and > 21%). Incidence rate ratios for the cranberry vs placebo groups were 0.76 (95% CI: 0.22, 2.60; p = 0.663) for those with < 10% probability, 0.73 (95% CI: 0.35, 1.53; p = 0.064) for those with 10% to 21% probability, and 0.58 (95% CI: 0.35, 0.97; p = 0.039) for those with > 21% probability.

The authors conclude that the results suggest that clinical predictors identify women with low and high risk of clinical UTI recurrence, which may be useful for design of clinical studies evaluating preventive therapies.

Barbosa-Cesnik *et al*. (2011) conducted a double-blind, placebo-controlled trial of the effects of cranberry on risk of recurring UTI among 319 college women presenting with an acute UTI (155 to **cranberry juice cocktail** and 164 to the placebo beverage). Participants were followed up until a second UTI or for 6 months, whichever came first. A UTI was defined on the basis of the combination of symptoms and a urine culture positive for a known uropathogen. The study was designed to detect a 2-fold difference between treated and placebo groups, as was detected in unblinded trials. Overall, the recurrence rate was 16.9% (95% confidence interval, 12.8%-21.0%), and the distribution of the recurrences was similar between study groups, with the active cranberry group presenting a slightly higher recurrence rate (20.0% vs 14.0%). The presence of urinary symptoms at 3 days, 1-2 weeks, and at ≥ 1 month was similar between study groups, with overall no marked differences. Among otherwise healthy college women with an acute UTI, those drinking 8 oz of 27% cranberry juice twice daily did not experience a decrease in the 6-month incidence of a second UTI, compared with those drinking a placebo.

The time to urinary tract infection (UTI) and the rates of asymptomatic bacteriuria and urinary P-fimbriated *Escherichia coli* during a 6-month period were compared in women ingesting cranberry vs placebo juice daily. Premenopausal women with a history of recent UTI were enrolled and randomized to 1 of 3 arms: 4 oz of cranberry juice cocktail (27% cranberry juice)/daily, 8 oz of **cranberry juice cocktail** daily, or placebo juice. Time to UTI (symptoms plus pyuria) was the main outcome. Asymptomatic bacteriuria, adherence, and adverse effects were assessed at monthly visits. Originally planned enrollment of 350 women to give a sample size of 210 in the cranberry group (105 taking 120 ml and 105 taking 240 ml) and 105 in the placebo group, allowing for a 10% dropout rate, to provide 80% power to detect an absolute reduction of 15% in the rate of symptomatic UTI recurrence. However, Study terminated in advance due to before the target sample size could be achieved because of administrative and budget issues. A total of 176 participants were randomized (120 to cranberry juice and 56 to placebo) and followed up for a median of 168 days. The cumulative rate of UTI was 0.29 in the cranberry juice group and 0.37 in the placebo group (P=0.82). The adjusted hazard ratio for UTI in the cranberry juice group vs the placebo group was 0.68 (95% confidence interval, 0.33-1.39; P=0.29). The proportion of women with P-fimbriated urinary *E. coli* isolates during the intervention phase was 10 of 23 (43.5%) in the cranberry juice group and 8 of 10 (80.0%) in the placebo group (P=0.07). The mean dose adherence was 91.8% and 90.3% in the cranberry juice group vs the placebo group. The authors conclude that cranberry juice did not significantly reduce UTI risk compared with placebo. (Stapleton *et al.*, 2012).

One hundred fifty sexually active women aged 21 through 72 years were randomized for one year to one of three groups of prophylaxis: (1) placebo juice + placebo tablets versus (2) placebo juice + cranberry tablets (**concentrated cranberry juice** (at least 1:30 parts concentrated juice)), versus (3) cranberry juice (pure unsweetened cranberry juice) + placebo tablets. Tablets were taken twice daily, juice 250 ml three times daily. Outcome measures were: (1) a >50% decrease in symptomatic UTI's per year (symptoms + >or= 100 000 single organisms/ml) and (2) a >50% decrease in annual antibiotic consumption. The authors report that both cranberry tablets statistically significantly decreased the number of patients experiencing at least 1 symptomatic UTI/year (to 20% and 18% respectively) compared with placebo (to 32%) ($p<0.05$) (Stothers, 2002).

Whole cranberry powder

This study tested whether **whole cranberry fruit powder** (CFP, declared total PACs was 0.56%, 250 mg/capsule, 1.4 mg of PACs/capsule) could prevent recurrent UTI in 182 women with two or more UTI

episodes in the last year. Participants were randomized to a cranberry (n = 89) or a placebo group (n = 93) and received daily 500 mg of cranberry for 6 months. The number of UTI diagnoses was counted. According to the authors, the intent-to-treat analyses showed that in the cranberry group, the UTIs were significantly fewer [10.8% vs. 25.8%, p = 0.04, with an age-"standardised" 12-month UTI history (p = 0.01)]. The Kaplan-Meier survival curves showed that the cranberry group experienced a longer time to first UTI than the placebo group (p = 0.04). Biochemical parameters were normal, and there was no significant difference in urinary phenolics between the groups at baseline or on day 180. In summary, results of this study showed that intake of 500 mg of cranberry fruit powder containing 2.8 mg of PACs/day for 6 months was associated with a reduction in incidence of recurrent UTIs (Vostalova *et al.,* 2015).

Caljouw *et al* (2014) performed a double-blind randomized placebo-controlled trial in 928 long-term care facilities residents (703 women, median age 84)). Cranberry and placebo capsules were taken twice daily for 12 months. The cranberry capsules contain 500 mg of the product, with 1.8% proanthocyanidins (9 mg)(plant part is not specified*). The placebo was indistinguishable in color, taste, and appearance, consisting of cellulose microcrystal colored red with azorubin.

Participants were stratified according to UTI risk (risk factors included long-term catheterization, diabetes mellitus, ≥1 UTI in preceding year). Main outcomes were incidence of UTI according to a clinical definition and a strict definition. Most participants had dementia (76%) or incontinence (64%). Therefore, a clean catch urine sample for culturing is often not available, making it impossible to diagnose UTI according to the strictest criteria.

The treatment effect of cranberry with respect to placebo was investigated using Cox proportional hazards models, expressed as hazard ratios (HRs).

In participants with high UTI risk at baseline (n = 516), the incidence of clinically defined UTI was lower with cranberry capsules than with placebo (62.8 vs 84.8 per 100 person-years at risk, P = .04); the treatment effect was 0.74 (95% confidence interval (CI) = 0.57– 0.97). For the strict definition, the treatment effect was 1.02 (95% CI = 0.68–1.55). No difference in UTI incidence between cranberry and placebo was found in participants with low UTI risk (n = 412). In the low-UTI-risk group, the incidence of UTI according to the clinical definition was 40.3 per 100 person-years at risk (95% CI = 30.0–50.5) for cranberry and 33.4 per 100 person-years at risk (95% CI = 24.2–42.5) for placebo (P = .30)
According to the authors, in LTCF residents with high UTI risk, taking cranberry capsules twice daily results in a 26% lower incidence of clinically defined UTI than with placebo, although no difference was found in UTI incidence according to a strict definition. Cranberry capsules may offer an opportunity to decrease the incidence of this common infection in high-UTI-risk LTCF residents by using a well-tolerated treatment.

Assessor's comment:

The effectiveness is questionable due to the most participants had dementia (76%) or incontinence (64%), therefore, a clean catch urine sample for culturing is often not available, making it impossible to diagnose UTI according to the strict criteria.
**In the Acknowledgement section of the article it is mentioned that Springfield Nutraceuticals B.V., Oud-Beijerland, the Netherlands, supplied the cranberry and placebo capsules. According to the internet this company sells Cranaxil cranberry concentrate (36:1) which contains the whole cranberry berry – flesh, peel, seed and juice.*

Burleigh *et al* (2013) conducted an observational study determine if consumption of sweetened, **dried cranberries** (SDC) decreases recurrent UTIs and whether this intervention would alter the heterogeneity, virulence factor (VF) profiles, or numbers of intestinal E. coli. Twenty women with

recurrent UTIs were enrolled in the trial and consumed one serving of SDC daily for two weeks. Clinical efficacy was determined by two criteria, a decrease in the six-month UTI rates pre- and post-consumption and increased time until the first UTI since beginning the study. Strain heterogeneity and virulence factor profiles of intestinal E. coli isolated from rectal swabs were determined by DNA fingerprinting and multiplex PCR, respectively. The numbers of intestinal E. coli eluted from rectal swabs pre- and post-consumption were also quantified.

Over one-half of the patients did not experience a UTI within six months of SDC consumption, and the mean UTI rate per six months decreased significantly. Kaplan-Meier analysis of infection incidence in women consuming SDC compared to patients in a previous control group showed a significant reduction in time until first UTI within six months. The heterogeneity, VF profiles, and prevalence of intestinal E. coli strains were not significantly different after cranberry consumption. According to the authors, results of this study indicate a beneficial effect from consuming SDC to reduce the number of UTIs in susceptible women. Because there were no changes in the heterogeneity or VF profiles of E. coli, additional studies are needed to determine the mechanism of action of SDC for reduction of UTIs.

Cranberry extract

An open label pilot study examined the ability of a concentrated cranberry preparation to prevent UTIs in women with a history of recurrent infections. Women between the ages of 25 and 70 years old were included with a history of a minimum of 6 UTIs in the preceeding year. The women took one capsule twice daily for 12 weeks containing 200 mg of a concentrated cranberry extract „standardised" to 30% phenolics (25% minimum proanthocyanidins). The total cranberry proanthocyanidin intake during the study was approximately 100 mg per day. Subjects were followed-up approximately 2 years later. All 12 subjects participated in the 12-week study and were available for follow up 2 years later. During the study none of the women had a UTI. No adverse events were reported. Two years later, eight of the women who continue to take cranberry, continue to be free from UTIs. They have all continued to take various cranberry supplements prepared by different manufacturers in doses ranging from 150 to 300 mg per day except for occasional days they missed. None of the subjects reported any adverse effects due to the supplement. Four of the patients stopped taking cranberry supplements for various unrelated medical reasons. One patient remained free of UTIs and two developed symptoms, which resolved upon resumption of cranberry supplementation. One patient developed a UTI confirmed by urinalysis and was treated with antibiotics (Bailey *et al*., 2007).

This pilot, registry study evaluates the prophylactic effects of oral supplementation with a „standardised" cranberry extract (Anthocran™) in patients with R-UTI, over a 2-month follow-up. Clinical outcomes were compared between patients on cranberry extracts and those who did not take this supplementation. In total, 22 females (Age: 39±4 years) completed the study in each of the two groups. The authors report that in the cranberry group, the reduction in the frequency of UTI episodes during the study period compared with the two months before the inclusion was 73.3% ($p < 0.05$). This figure was 15.4% in the control group ($p < 0.05$; $p = 0.012$ vs cranberry group). Seven (31.8%) subjects in the cranberry group were symptom-free; no patient was symptoms free in the control group ($p < 0.05$). The mean duration of UTI episodes was 2.5±1.3 days in the cranberry group, compared with 3.6±1.7 days in subjects not on cranberry ($p < 0.05$). Three subjects (13.6%) in the cranberry group and 8 (36.3%) in the control group required medical consultation for UTI symptoms ($p < 0.05$). Urine evaluation was completely negative in 20/22 subjects in the Cranberry group (90.9%) and in 11 control subjects (50.0%; $p < 0.005$). No adverse events were observed (Ledda A *et al*., 2015).

Occhipinti *et al* (2016) conducted a pre-clinical double-blind controlled study with Cranberry Extract (plant part is not specified in the article) with a high content of A-type proanthocyanidins. A balanced group of female (n=60, age ranging from 19 to over 51 years) and male volunteers (n=10 over 51

years), with at least 2 culture-documented symptomatic UTIs in the calendar year prior to recruitment, was divided into two groups. The experimental group (5 males and 30 females) received 1 capsule containing cranberry extract (36 mg PACs-A) twice per day (morning and evening) for 7 days, and the placebo group (5 males and 30 females) was given the same number of capsules with no PACs. At the end of the treatment period, a urine sample was sent for urine analysis and urine culture. Fisher's exact tests on the tabulated frequencies was performed to assess the effect of the treatments.

After 7 days of cranberry extract and placebo administration, a contingency table was calculated based on recovered vs. not recovered volunteers.

Table 3. Contingency table.

Variables	Placebo	Oximacro©
Not recovered	35	7
Recovered	0	28

Fisher's Exact Test: $P < .001$

This table showed a significant difference (Fisher's exact test: P < .001) between the two groups. Most of the placebo group unable to recover from UTI. Eventually, all placebo volunteers had to be treated with antibiotics (Monuril®, trometamol salt of fosfomycin) to reduce pain.

112 mg cranberry extract (equivalent of 36 mg PACs-A) twice per day for 7 days was significantly effective in reducing the total urobacterial CFU counts in both the female and male groups with respect to placebo (SD difference = 51688; df = 34, t = -10.27; Dunn-Sidak Adjusted P < .001, Bonferroni Adjusted P < .001). The age ranges were unaffected by treatment with the sole exception of the 31-35 year age range in the female group. This group did not differ in baseline characteristics with respect to the other age groups; thus, the reason for the reduced effect in this group requires further investigation. A literature search on age-related responses to cranberry treatment did not provide any reported cases, although further studies will focus on this aspect.

One hundred and thirty-seven women with two or more antibiotic-treated UTIs in the previous 12 months were randomized to receive either 500 mg of **cranberry extract** (n=69) or 100 mg of trimethoprim (n=68) for 6 months. Thirty-nine of 137 participants (28%) had an antibiotic-treated UTI (25 in the cranberry group and 14 in the trimethoprim group); difference in proportions relative risk 1.616 (95% CI: 0.93, 2.79) p=0.084. The time to first recurrence of UTI was not significantly different between the groups (p=0.100). The median time to recurrence of UTI was 84.5 days for the cranberry group and 91 days for the trimethoprim group (U=166, p=0.479). There were 17/137 (12%) withdrawals from the study, 6/69 (9%) from the cranberry group and 11/68 (16%) from the trimethoprim group (p=0.205), with a relative risk of withdrawal from the cranberry group of 0.54 (95% CI: 0.19, 1.37). (McMurdo *et al.*, 2009).

Beerepoot *et al* (2011) conducted a double-blind, double-dummy noninferiority trial, in which 221 premenopausal women with recurrent UTIs were randomized to 12-month prophylaxis use of trimethoprim-sulfamethoxazole (TMP-SMX) (n=95), 480 mg once daily, or cranberry extract, 500 mg twice daily (n=104). Primary end points were the mean number of symptomatic UTIs over 12 months, the proportion of patients with at least 1 symptomatic UTI, the median time to first UTI, and development of antibiotic resistance in indigenous Escherichia coli.

After 12 months, the mean number of patients with at least 1 symptomatic UTI was higher in the cranberry than in the TMP-SMX group (4.0 vs 1.8; P=.02), and the proportion of patients with at least 1 symptomatic UTI was higher in the cranberry than in the TMP-SMX group (78.2% vs 71.1%). Median time to the first symptomatic UTI was 4 months for the cranberry and 8 months for the TMP-SMX group. After 1 month, in the cranberry group, 23.7% of fecal and 28.1% of asymptomatic bacteriuria *E coli* isolates were TMP-SMX resistant, whereas in the TMPSMX group, 86.3% of fecal and 90.5% of asymptomatic bacteriuria *E coli* isolates were TMP-SMX resistant. Similarly, we found increased resistance rates for trimethoprim, amoxicillin, and ciprofloxacin in these *E coli* isolates after 1 month in the TMP-SMX group. After discontinuation of TMP-SMX, resistance reached baseline levels after 3 months. Antibiotic resistance did not increase in the cranberry group. Cranberries and TMP-SMX were equally well tolerated.

Table 5: Clinical studies on humans, in **Prevention of UTI**

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
Maki *et al.*, 2016	double-blind placebo contr. multicentre 24 weeks	240-mL serving of **cranberry juice** cocktail (27% cranberry juice)/d or placebo study beverage/d	Women, 20–70 y (n=185) or a placebo (n=188) 86% of subjects completed the treatment period in both groups. The mean age was 40.9 y, and characteristics were similar in both groups.	a recent history of a UTI, which was defined as 2 episodes of a UTI that were treated by a health care professional in the past year (self-report) of which 1 UTI had been treated 6 months of the screening visit	Primary UTI incidence density: Verum: 39 episodes Placebo: 67 episodes 39% reduction in Clinical UTI episodes. incidence ratio: 0.62;95% CI: 0.42, 0.92 P=0.017	ITT (observation time censored at the time that the study product was discontinued for subjects who did not complete the full	The results of this clinical trial indicate that the daily consumption of 240 ml cranberry juice cocktail reduces the frequency of clinical UTI in women with the self-reported

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					UTI with pyuria reduced by 37% incidence ratio:0.63; 95% CI: 0.40, 097; P=0.037 UTI was diagnosed by the investigator on the basis of ≥1 of the following symptoms: dysuria, urinary frequency, urinary urgency, or suprapubic pain in the absence of other potential etiologies such as		history of recent UTI. However, there was no difference between the groups observed for microbiologically positive UTI.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					vaginal infection or discharge. Secondary: No differences in time to first clinical UTI with pyuria and UTI with microbiological positivity or positive for E. coli		
Stapleton *et al.,* 2012 Prevention of UTI	randomized doble-blind placebo-controlled three-arms	4 oz (120 ml) of cranberry juice cocktail (27% **cranberry juice**)/daily, 8 oz (240 ml) of cranberry	A total of 176 Premenopausal women 18 to 45 years of age	with a history of one or more clinician-diagnosed	Primary: time to a symptomatic UTI event that met criteria for clinical or culture-confirmed UTI.time to first UTI: hazard ratio	2 sided .05 significance level in ITT population, continuous variables: t	Cranberry juice did not significantly reduce culture-confirmed

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
	multi-centre 6 months	juice cocktail daily, or matching placebo juice provided by Ocean Spray Cranberries Inc	(in 1.1.1 ratio: 120 to cranberry juice and 56 to placebo) The mean age was 25 years,	UTIs in the past 12 months were eligible to participate. If the urine culture result was positive (≥103 CFU/mL of a uropathogen), the episode was catego- rized as culture-confirmed UTI. If the urine culture result was negative, alternate diagnoses were excluded, and if	for UTI in the cranberry vs placebo group 0.78 (95% CI, 0.43-1.41; P=.41). the rates of asymptomatic bacteriuria and urinary P-fimbriated E. coli 33/120, (27.5%) Placebo: 17/56, (30.4%) (P=0.70). more than one UTI:	test, caterogical variables: χ^2 test Dose-specific analyses were not performed because the combined dose comparisons were not significant.	UTI risk compared with placebo. The desired sample size was not achieved due to study terminated in advance.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
				symptoms resolved with therapy, the episode was categorized as a clinical UTI. Asymptomatic bacteriuria was defined as 105 CFU/mL or more of a uropathogen in a woman without symptoms of UTI	verum. 10 (8.3%) Placebo : 4 (7.1%)		

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
Barbosa-Cesnik *et al.,* 2011	prospective, double-blind, placebo controlled, randomized	drinking 8 oz (240 ml) of 27% **cranberry juice** (Ocean Spray) twice daily (mean proanthocyanidin concentration of 112 mg per dose) or placebo for 6 months.	319 college women presenting with an acute UTI, Two hundred thirty (72%) of 319 participating women completed the entire protocol. 155 cranberry juice, 164 placebo.	women presenting with an acute UTI	Primary endpoint was a culture-confirmed recurrent UTI or for 6 months, whichever came first. Hazard ratio of cranberry versus placebo (1.4; 95% CI, 0.8–2.4). Cumulative incidence rate, 19.3% treatment group vs 14.6% placebo group; log-rank P= 0.21 Overall, the recurrence rate was 16.9% (95% confidence interval,	risk of UTI: ITT analysis	No decrease in the 6-month incidence of a second culture-confirmed UTI, compared to placebo

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
			Eighty-four were lost to follow-up or dropped out voluntarily, and 5 became pregnant and were excluded		12.8%–21.0%), and the distribution of the recurrences was similar between study groups, (20.0% vs 14.0%). Statistical significance was not achieved. The presence of urinary symptoms at 3 days, 1–2 weeks, and at >1 month was similar between study groups, with overall no marked differences.		

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
Stothers, 2002	randomized, double-blind, controlled study 12 months	1 tablet of concentrated **cranberry juice** (at least 1:30 parts concentrated juice twice daily 250 ml of pure unsweetened cranberry juice three times per day	Women Placebo group (n=50, mean age 43) tablet group (n=50 mean age 40), juice group (n=50, mean age 44)	at least two symptomatic, single-organism, culture positive UTI in the prior calendar year, but currently free of UTI on urinalysis and culture	In the primary endpoint, i.e. a >50% decrease in symptomatic UTI's per year (symptoms + >or= 100 000 single organisms/ml), both cranberry juice and cranberry tablets statistically significantly decreased the number of patients experiencing at least 1 symptomatic UTI/year (to 20% and 18% respectively) compared with placebo (to 32%) ($p<0.05$)	ANOVA for statistical significance	In this small study, a small effect compared to placebo on culture-confirmed UTI was found

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
McMurdo *et al.,* 2009	comperativ e study	500 mg of **Cranberry extract** (n=69) or 100 mg of trimethoprim (n=68) daily for 6 months	137 women aged >45 years mean cran 62.6, trim63.3	two or more antibiotic-treated UTIs in the previous 12 months	Primary endpoints: the proportion of participants experiencing a recurrence of an antibiotic-treated UTI: 25 in cranberry group, 14 in the trimethoprim group, p=0.084, 95% CI: 0.93-2.79 the time to first recurrence of culture positive UTI: not significant between the groups, 84.5 and 91 days, p=0.1	Time to first recurrence of infection is presented as a Kaplan–Meier curve and differences between the groups were assessed using the log-rank test.	Proportion of participants experiencing a recurrence of an antibiotic-treated UTI and the time to first recurrence of UTI was not significantly different between the groups.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					withdrawals: 9 and secondary endpoints 16%, p=0.205, relative risk of cranberry treatment 0.54 (95% CI: 0.19-1.37)		There was no placebo group.
Sengupta *et al.,* 2011	randomized double blind, controlled, dose-dependent clinical trial	proanthocyanidin „standardised" whole cranberry powder (PS-WCP, „standardised" to 1.5% proanthocyanidins using a proprietary	Women 60 female subjects between 18-40 years of age was conducted. untreated control group	Women with a history of painful urination and frequency, passing blood in the urine or pain in the suprapubic area, and with a negative	at the end of the study there was significant reduction ($p<0.05$) at day 90 in the subjects positive for E. coli as well as symptomatic relief in both the high dose and low dose treatment groups,	F-test on positive cases, multiple comparison tests, pair-wise t-tests for the baseline and other time	Small dose-finding study with untreated control group with a lower number of participants than the

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
		Process, 14.4 mg/g PACs,). 90-day three groups: control, a low dose (250 mg twice daily) and a high dose (500 mg twice daily, n=23) treatment group.	(n=16), a low dose (500 mg daily, n=21) and a high dose (1000 mg daily, n=23) treatment group.	pregnancy test were included	compared to baseline evaluation, 10% increase in the control group 25% decrease in the low dose 50 % decrease in the high dose group	periods, ANOVA	treatment groups. Also, the presence of E. coli in the culture of urine samples at baseline was lower in the control group than in the two cranberry groups
Vostalova *et al.,* 2015	randomized, double-blind,	**2x250 mg cranberry fruit powde**r daily (100%	182 women were	Women from 18 to 75 years: Symptomatic UTI at baseline; A	UTI significantly lower in the cranberry group than in the placebo	ITT binomial regression model.	Treatment with 500 mg powder (PAC

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
	placebo controlled study	fruit from NATUREX-DBS (Sagamore, MA, USA) PAC content 0.56% for 6 month or placebo	randomized to the cranberry (n=89, mean age 35) or placebo (n=93, mean age 38) groups. Seventeen women did not complete the study	history of recurrent symptomatic UTIs (defined as a medical history of at least two symptomatic UTI episodes treated with antibiotics in the previous 12 months) The clinical diagnosis of aUTI was based on bacteriuria plus the manifestation of at least one of the following symptoms: pollakiuria (strong, persistent urge to	group 10.8% (9/83) vs. 25.8% (24/93)(p=0.04), corresponding to relative risk reduction of 58% in the cranberry group relative to the placebo group		content 2.8 mg) daily for 6 months showed relative risk reduction of 58% compared to placebo. The recurrence of UTI was medically diagnosed and confirmed microbiologically.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
				urinate and passing frequent, small amounts of urine), burning sensation on micturition, hematuria, turbid or malodorous urine, subpelvic pain, pruritus, fever and dysuria.			
Bailey *et al*., 2007	open, pilot study	one capsule twice daily for 12 weeks containing 200 mg of a concentrated **cranberry extract** „standardised" to 30% phenolics (25%	12 Women between the ages of 25 and 70 years old	with a history of a minimum of 6 UTIs in the proceeding year.	No recurrence of UTI in women with a history of recurrent infections during the study period of 12 weeks	Not mentioned.	This pilot study is considered not relevant due to the size of the study, no

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
		minimum proanthocyanidins).					control group included and the open design.
Efros *et al.,* 2010	Open dose-finding study	A concentrated cranberry liquid blend at 15, 30, 45, 60, and 75 mL daily for 12 weeks. 3875 mg (cranberry concentrate [4:1], ascorbic acid, D-mannose, fructo-oligosaccharides, and bromelain) per 30 mL	Women (average age 46.5 ± 12.8 years	Women with a history of 2.78 ± 0.73 rUTIs <6 months.	primary: efficacy: 2/23 (9.1%) rUTI safety: 75 ml/d ADR in 3/3 patients maximal tolerated dose: 60 mL/d secondary:	descriptive statistics mean ±SD Student t-test	This dose-finding study is considered not relevant due to the size of the study, no control group included and

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					QoL: AUA Symptom Index decreased to week 4 p=0.39, to week 12 p=0.45		the open design.
Avorn at al. 1994	randomize, double blind, placebo-controlled	300 ml/d cranberry juice, (commercially available cranberry beverage) 6-month	153 women mean age 78.5 year 63 patients in the cranberry group and 61 patients in the placebo group completed the study	women older than 65 years	Bacteriuria with pyuria was found in 28.1% of urine samples in the placebo group and only 15.0% in the group randomized to cranberry beverage. The difference was not present in the first month, but appeared most strikingly between month 1 and 2 and remained fairly stable	logistic regression model with SEs (effect on bacteriuria with pyuria), 95% CI	Reduced frequency in bacteriuria with pyuria compared to placebo. The effect on bacteriuria and pyuria concurrent with the subject's reporting

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					throughout the rest of the trial (6 months). OR 0.42 for bacteriuria with pyuria in treatment group (95% CI 0.23-0.76, P=0.004). The effect persisted when taking into account baseline difference in previous UTI: 6 months before randomisation 0.53, P=0.049) 12 months before randomisation 0.48 P=0.01		urinary tract symptoms was not statistically significant. Baseline data different between treatment group and placebo for rate of UTI previous 12 months i.e. lower rate in subjects

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					In addition, out of the 473 urine samples collected in the cranberry group 20 (4%) had bacteriuria and pyuria concurrent with the subject's reporting urinary tract symptoms, compared with 37 (7%) of 498 urine samples in the placebo group, however, the result was not statistically significant.		randomised to cranberry treatment.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
Beerepoot *et al*. 2011	double-blind, double-dummy, non-inferiority trial 12-month prophylaxis	cranberry extract (Cran Max, Proprietary Nutritionals, Inc, Kearny, New Jersey) 500 mg capsule twice daily vs trimethoprim-sulfamethoxazole (TMP-SMX) 480 mg once daily	221 premenopausal women TMP-SMX n=95 cranberry n=104	rUTI	symptomatic UTI higher in cranberry group (4.0 vs 1.8; p=0.02) the proportion of patients with at least 1 symptomatic UTI was higher in the cranberry than in the TMP-SMX group (78.2% vs 71.1%). Median time for the first symptomatic UTI 4 vs 8 months	linear regression	TMX-SMX more effective the cranberry capsule to prevent rUTI.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					TMP-SMX resistance was higher in the TMX-SMX group		
Bianco *et al*. 2012	multicentre, randomized, double-blind, dose-finding, placebo-controlled pilot study	cranberry capsule containing 36 mg proanthocyanidin (PAC), 1, 2 or 3 capsules daily or placebo for 28 days	nursing home residents women, age ≥65 years stratified by presence or absence of baseline bacteriuria 20 participants are randomized by	women with history of UTI,	4 weekly analysis of 320 urine samples E. coli bacteriuria was reduced, bacteriuria with other pathogens did not reduced the effect of two or three capsules was comparable	SAS 9.22 statistical software	Small, dose-finding pilot study. Although the E.coli bacteriuria was reduced, but the difference between the placebo and the treated

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
			strata by each arm				groups cannot be considered clinically relevant.
Burleigh *et al*. 2013	self-controlled 6 months study	42 g sweetened, dried cranberry/d for two weeks	20 women 3 dropped out	defined at least three UTIs in the past year (with two UTIs in the past 6 months)	six-month UTI rates decreased significantly compared to pre-consumption significant reduction in time until first UTI, virulence factor profiles of E.coli did not change incidence of UTI	two-tailed t-test	No clinical relevance due to methodological deficiencies (e.g. self-controlled study;

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
					(according to the clinical and strict UTI definition)		antibacterial activity was not proved).
Caljouw *et al*. 2014	double-blind, randomized, placebo-controlled, multicentre trial 12 months	cranberry capsules 500 mg with 1.8% (9mg) proanthocyanidin twice a daily for 12 months	928 long-term care facility (LTCF) residents, 703 women, >65 years old, median age 83 years	LTCF resident, >65 years old, stratified according to baseline UTI low or high risk 400 low UTI risk, 516 high UTI risk	in high UTI-risk group the incidence of clinically defined UTI decreased significantly (p=0.03), but there was no such effects for strictly defined UTI (p=0.91). There was no difference between the two groups in the low UTI-risk group.	Student t-test, chi-square test 95% CI	the effectiveness is questionable due to the most participants had dementia (76%) or incontinence (64%). Therefore,

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
					The incidence of rUTI did not differ significantly		a clean catch urine sample for culturing is often not available, making it impossible to diagnose UTI according to the strictest criteria.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
Dignam *et al*. 1998	retrospective cross-sectional and longitudinal cohort study	cranberry juice 4 oz (120 ml) /d 6 cranberry capsules/d 33 months	538 participants in the cross-sectional study and 113 residents in the longitudinal study treatmen period: 8 months 73% female and 23 % male	long-term care facility residents	cross-sectional study: rUTI rate decreased significantly p=0.0008 longitudinal study: rUTI rate NS	Student t-test	Efficacy was reported in the cross-sectional study but not in the longitudinal study

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
Ledda *et al*. 2015	Pilot, registry study	one capsules with cranberry extract	cranberry treatment + lifestyle advice: 22 female median age 39±4 years 22 female with only lifestyle advice median age 39±3 years	subjects with at least 3 rUTI in the past year	rUTI rates in the cranberry group was significantly lower than in control group, p=0.012 the duration of UTI episodes was also reduced, p<0.05	Student t-test or Mann-Whitney U-test	This pilot study is considered not relevant due to the size of the study and the open design
McMurdo *et al*. 2005	randomized, double-blind, placebo-	300 ml low calorie cranberry juice matching placebo	hospitalized 376 older patients (men and women)	age >60 years	symptomatic UTI rates adherence	Student t-test	there was no statistical difference between the

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
	controlled study	35 days	randomized in two groups		antibiotic use	chi squared test Kaplan Meier analysis	cranberry an placebo group
Occhipinti *et al*. 2016	Randomized clinical study Prevention of UTIs in volunteers	Cranberry extract „standardised" to 360 mg/g of PACs content. Group (1) Cranberry extract containing 36 mg PACs-A was administered twice a day (total 72 mg of PACs-A /day) Group (2) Placebo	1)Cranberry group (35): females, n=30, males, n=5 1)Placebo group (35): females, n=30, males n=5	Inclusion criteria: at least 2-culture-documented symptomatic UTIs in the previous year. Positive UTI was recognized with a uropathogenic bacterium at 105 colony forming unit (CFU/ml).	After 7 days of cranberry extract administration, a significant difference was found between the placebo and cranberry groups for both females (Mann-Whitney U-test = $p<0.001$) and males (Mann-Whitney U-test =p =0.016). Colony forming unit/mL showed	Fisher's exact test. Non parametric ANOVA was used according to the sex and age categories.	This is a small and short study

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form;** **Dosage** **Regimen;** **Route of** **Administration** **Duration of treatment**	**Number of** **Subjects (including age, sex, drop out)**	**Healthy** **Subjects or** **Diagnosis of** **Patients** **(inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score** e.g. Jadad score	**Comments on clinical relevance of results**
		Duration of treatment: 7 days	Age range in females varied from 19 - >51 years, in males over 51 years Drop out: cranberry group – 7,,placebo group - 16	Symptoms: pain before, during, or after micturition; increased frequency of micturition; pain in abdomen; haematuria; (fever > 37.9°C or 1.5°C above baseline, temperature, chills, nausea, and vomiting).	a significant difference ($p < 0.001$) between the cranberry and the placebo groups ($p<0.001$).	Post-hoc Dunn-Sidak and Bonferroni tests.	

Meta-analysis

Jepson *et al*., 2012, is the third update of their review first published in 1998 and updated in 2004 and 2008, with the objective to assess the effectiveness of cranberry products in preventing UTIs in susceptible populations. This updated review includes a total of 24 studies. Prior to the current update it appeared there was some evidence that cranberry juice may decrease the number of symptomatic UTIs over a 12 month period, particularly for women with recurrent UTIs. The addition of 14 further studies suggests that cranberry juice is less effective than previously indicated. Although some of small studies demonstrated a small benefit for women with recurrent UTIs, there were no statistically significant differences when the results of a much larger study were included (Jepson *et al*., 2012).

In 2017, Luis et al. published a systematic review on the effectiveness of the use of cranberry in reducing the UTI frequency. 25 studies were included in this systematic review, some of them published after the meta-analysis by Jepson et al., 2012. The included studies are very heterogenic in the product composition, posologies, and study population. The authors conclude that there is strong evidence that cranberries may decrease number of UTIs, especially in patients with recurrent UTIs.

Recommendations from Urological Associations

The American and Canadian Urological Associations for Recurrent Uncomplicated Urinary Tract Infections in Women: AUA / CUA / SUFU Guideline (Anger et al. 2019) allow the use of cranberry by classifying it as: "C" (low body of evidence). Cranberry (according to the Conditional Recommendation and Evidence Level: Grade C) may be offered as prophylaxis, including oral juice and tablet formulations, as there is not sufficient evidence to support one formulation over another. However, according to the guidelines of the European Association of Urology (Bonkat *et al*. 2018) due to contradictory results of clinical trials, no recommendation on the daily consumption of cranberry products can be made.

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

Children

In clinical trial of Ledda *et al.* (2017), 36 otherwise healthy subjects in juvenile age (between 12 and 18 years of age) suffering by recurrent UTIs were enrolled. Participants received either a standard management (SM) (control group, n=17) or standard management associated with an oral daily supplementation (supplementation group, n=19). Oral supplementation consisted in one capsule containing 120 mg of cranberry extract, „standardised" to 36 mg proanthocyanidins, for 60 days. The effectiveness in the prevention of UTIs was determined by: the number of UTIs evaluated two months before the inclusion in the registry and during the supplementation period; the number of symptom-free subjects during the registry period. Safety considerations and measurement of adherence to treatment were also performed.

The two groups were comparable for age, gender distribution, the days of follow-up and also for the number of UTIs before inclusion. The mean number of UTIs observed during the registry in the supplemented group (0.31±0.2) was significantly lower compared to the control group (2.3±1.3) and to the mean number of UTIs assessed before inclusion (1.74±1.1) (p-value = 0.0001 for both). Moreover, 63.1% of supplemented subjects was symptom-free during the registry period, whereas 23.5% sub jects were asymptomatic in the control group (p-value <0.05) (Ledda et al., 2017).

In a study performed by Afshar *et al* (2012) a total of 40 children were randomized to receive daily 2 cc/kg cranberry juice containing 37% PAC vs cranberry juice with no proanthocyanidin for a 1-year period. The study was powered to detect a 30% decrease in the rate of symptomatic urinary tract infection with type I and II errors of 0.05 and 0.2, respectively. Toilet rained children up to age 18 years were eligible if they had at least 2 culture documented nonfebrile urinary tract infections in the calendar year before enrolment. Patients with anatomical abnormalities (except for primary vesicoureteral reflux) were excluded from study. Subjects were followed for 12 months. The participants, clinicians, outcome assessor and statistician were all blinded to treatment allocation.
Of the children 39 girls and 1 boy were recruited. Mean and median patient age was 9.5 and 7 years, respectively (range 5 to 18). There were 20 patients with comparable baseline characteristics randomized to each group.
After 12 months of follow-up the average incidence of urinary tract infection in the treatment group was 0.4 per patient per year and 1.15 in the placebo group (p = 0.045), representing a 65% reduction in the risk of urinary tract infection.

Salo (2012) presented a double-blind randomized placebo-controlled trial which as performed in 7 hospitals in Finland. A total of 263 children treated for UTI were randomized to receive either cranberry juice (n=129) or placebo (n=134) for 6 months. Eight children were omitted because of protocol violations, leaving 255 children for the final analyses. The children were monitored for 1 year, and their recurrent UTIs were recorded.
Twenty children (16%) in the cranberry group and 28 (22%) in the placebo group had at least 1 recurrent UTI (difference, - 6%; 95% confidence interval [CI], - 16 to 4%; P= 0.21). There were no differences in timing between these first recurrences (P= .32). The intervention did not significantly reduce the number of children who experienced a recurrence of UTI. Episodes of UTI totaled 27 and 47 in the cranberry and placebo groups, respectively, and the UTI incidence density per person-year at risk was 0.16 episodes lower in the cranberry group (95% CI, -.31 to -.01; P = .035). However, the children in the cranberry group had significantly fewer days on antimicrobials (- 6 days per patient-year; 95% CI, -7 to - 5; P<.001).

Assessor's comment:

There are numerous clinical studies performed with cranberry juice/extracts in children of different age groups. However, from the information obtained from member states, there are no products authorised in EU for more than 10 years for the prevention/treatment of uncomplicated UTI in children. Hence, a well-established use monograph cannot be established for the use in children.

4.4. Overall conclusions on clinical pharmacology and efficacy

Lots of articles investigated the effect of cranberry on pH of the urine suggesting the benzoic acid (Blatherwick, 1914, Blatherwick *et al*., 1923) or the quinic acid (Fellers 1933, Kahn *et al* 1967, Kinney 1979) caused large amounts of hippuric acid to be excreted in the urine (Bodel *et al* 1959), which then acted as an antibacterial agent. As the urine acidifying properties could not be proved, other mechanism of action was investigated behind the traditional use. Sobota (1984) investigated other possible mechanism of action, *antiadherence activity in the urine,* behind the traditional use. Several *ex vivo/in vitro* studies suggest that cranberry constituents or metabolites present in human urine inhibit bacterial adhesion to uroepithelial cell (antiadhesive activity). The mode of action is comprehensively discussed in section 3.1.4.

A number of randomised, placebo-controlled, and double-blind clinical trials have been conducted for the evaluation of efficacy of cranberry juice in the prevention of recurrent UTI in women. In the studies by Maki *et al*., 2016, Stapleton *et al*., 2012, Barbosa-Cesnik *et al*., 2011, and Stothers *et al*., 2002, cranberry juice in the daily posology of 120-750 ml juice daily was compared to placebo during approximately 6-12 months. However, the studies report both positive and negative outcome and a coherence of scientific assessment is lacking.

In the randomised, controlled and double-blind studies by Vostalova *et al*., 2015, Caljouw *et al*., 2014, Hout *et al*., 2014, Beerepoot *et al*., 2011, and Mc Murdo *et al*., 2009, dried cranberry preparations in doses of 500 mg daily was compared to placebo or antibiotics during 6-12 months. Also for these studies both positive and negative outcome is reported and a coherence of scientific assessment is lacking.

There is also inconsistency between the studies regarding the criteria that were used to diagnose a UTI. It should be a clear definition of "breakthrough" cases in the studies and microbiologically confirmed UTI is considered the highest evidence of a breakthrough.

Additional studies found in the literature have been performed with other cranberry preparations or with insufficiently described cranberry preparation. Some studies included only elderly women in in long-term care facilities. Furthermore, several additional studies show poor study design e.g. small numbers of subjects, the lack of a control group, or not double blind, short duration of study, as well as high drop-out rate in some of the studies, that considerably limit their value as a source of evidence to substantiate efficacy in prevention of recurrent UTI.

The meta-analysis by Jepson *et al*., 2012 and Luis *et al*., 2017 include studies with heterogenous cranberry preparations and posologies. Not only did the included studies utilize different administration vehicles (e.g., tablets, capsules, juices) but also the dosage and concentration of the cranberry products were never accurately and properly determined. Due to the substantial variability across trials in the quantitative and qualitative chemical composition of the cranberry products, it is difficult to draw valid conclusions about the effectiveness of cranberry products, as was also pointed out by the authors of the latest Cochrane study i.e. Jepson at al. 2012.

The American and Canadian Urological Associations for Recurrent Uncomplicated Urinary Tract Infections in Women: AUA / CUA / SUFU Guideline (Anger *et al*., 2019) allow the use of cranberry by classifying it as: "C" (low body of evidence). Cranberry (according to the Conditional Recommendation and Evidence Level: Grade C) may be offered as prophylaxis, including oral juice and tablet formulations, as there is not sufficient evidence to support one formulation over another. However, according to the guidelines of the European Association of Urology (EAU) (Bonkat *et al*., 2018, 2020) due to contradictory results of clinical trials, no recommendation on the daily consumption of cranberry products can be made.

HMPC shares the opinion of EAU that the results of the clinical studies is inconsistent and concludes that the requirements for well-established medicinal use according to Article 10a of Directive 2001/83/EC is considered not fulfilled.

5. Clinical Safety/Pharmacovigilance

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

Adverse events that occurred in 5% of subjects in either treatment group included headache [cranberry group: n=16 (8.6%); placebo group: n=12 (6.4%)], sinusitis [cranberry group: n=10 (5.4%); placebo group: n=6 (3.2%)], and upper respiratory infection [cranberry group: n=13 (7.0%); placebo group: n=13 (6.9%)]. One subject in the cranberry group had a serious adverse event (chest pain), and 4 subjects in the placebo group had a serious adverse event (ischemic colitis leading to septic shock, miscarriage, in-patient hospitalization for appendicitis, and surgery for a rectal prolapse). All serious adverse events were classified as either unrelated or unlikely to be related to the treatment (Maki *et al.,* 2016).

No serious adverse events occurred in either of the study groups. Minor adverse effects included primarily gastrointestinal (constipation, heartburn, loose stool), vaginal (itching, dryness), and other (migraine) symptoms. The proportions of women who reported minor adverse effects were 29 of 120 (24.2%) in the cranberry juice group and 7 of 56 (12.5%) in the placebo group (P=0.07). Three women in the cranberry group stopped taking the study product because of gastrointestinal symptoms thought to be related to the study product (Stapleton *et al.,* 2012).

Serious adverse events occurred equally in both groups, and none were deemed to be attributable to treatment after review by the data safety monitor (Barbosa-Cesnik *et al.,* 2011).

There were 17/137 (12%) withdrawals from the study, 6/69 (9%) from the cranberry group and 11/68 (16%) from the trimethoprim group (P. 0.205), with a relative risk of withdrawal from the cranberry group of 0.54 (95% CI: 0.19, 1.37). The reasons were as follows: for the cranberry group, gastrointestinal upset n=4; increased nocturia n=1; sensitive swollen nipples n=1 and the trimethoprim group, gastrointestinal upset n. 4; itch/rash n=3; lost to follow-up n=2; restless legs n=1; increased lethargy n=1. While gastrointestinal upsets were equally common in both groups, itch/rash and loss to follow-up occurred more commonly in the trimethoprim group. Other adverse events were similar between the groups (McMurdo *et al.,* 2009).

Dose escalation proceeded to 75 mL/d, at which point 3 of 3 patients developed adverse events, including diarrhea, headache, and heartburn at the 75 mL/d dose. Therefore, the maximal tolerated dose of UTI-STAT with Proantinox was set at 60 mL/d. All other treatment related adverse events in the 15-60 mL regimens were mild to moderate in severity. The complaints were primarily gastrointestinal in nature and resolved either when the study drug was taken with food or spontaneously. No significant correlations were seen between the pre- and postmenopausal women

with regard\ to the frequency of the reported adverse events. Of the 5 women with nonanalyzable data, 1 discontinued because of a dislike of the taste (group 4, 60 mL), 3 women were lost to follow-up (1 at the 15 mL/d dose, 1 at the 45 mL/d dose, and 1 at the 60 mL/d dose), and 1 withdrew because of the presence of another bacterial infection (sinus) (Efros *et al.,* 2010).

In the juice group, symptoms of reflux were reported by 3 patients, 2 of whom dropped out due to this problem. Complications reported in the tablet group were: mild nausea (4 patients) and increased frequency of bowel movements (1 patient). None of these complications requested discontinuation of the treatment (Stothers, 2002).

There were no serious adverse events during the study period. Very mild adverse events occurred during the study as detailed below, and no subjects withdrew from the study due to adverse effects. General weakness and lower abdominal pain were recorded in three and one patients, respectively in the 500 mg/day and 1000 mg/day PS-WCP groups. A mild fever (99.5 °F to 100 °F) was reported by three participants in the 1000 mg PS-WCP group. Two participants in this group complained of heartburn at the 60th day visit during the study. Two participants in the untreated control group reported "stomach burn" and general weakness during the follow-up period. Overall, it was evident that this cranberry product was well accepted by all participants. Furthermore, supplementation of PS-WCP over a period of 90 days did not result in antibiotic resistance among the bacteria detected, which substantiates the safety of PS-WCP over prolonged use (Sengupta *et al.,* 2011).

The remaining 182 eligible women were enrolled and were randomized to the cranberry (n=89) or placebo (n=93) groups. Seventeen women did not complete the study, seven (7%) in the placebo group and ten (11%) in the cranberry group. Reasons for not completing the study included loss to follow-up, voluntary withdrawal (n=14) or pregnancy (n=3) (Vostalova *et al.,* 2015).

5.2. Patient exposure

A considerable patient/consumer exposure should be taken into consideration as cranberry is used as a flavouring agent in the food area. Cranberry's safety is well established in food use throughout the world and is formally generally recognized as safe (GRAS).

5.3. Adverse events, serious adverse events and deaths

According to Upton and Brendler (2016) most of the dverse effects reported in clinical studies (see also section 5.1) belonged to the 'Gastrointetinal disorders' of Sytem orgam class: "Nausea (Basu *et al*. 2011); nausea, vomiting, and/or diarrhoea (Juthani-Mehta *et al*. 2010); nausea, diarrhoea, constipation, and rash (Lee *et al*. 2007); nausea, diarrhoea, constipation, rash or urticaria, vomiting, and vaginal complaints (Beerepoot *et al*. 2011); mild nausea, and increased frequency of bowel movements (Stothers and Stothers 2001); gastrointestinal upset including nausea, vomiting, and diarrhoea (Wing *et al*. 2008); constipation, heartburn, loose stool, vaginal itching and dryness, migraine (Stapleton *et al*. 2012); gastrointestinal upset, increased nocturia, sensitive and swollen nipples (McMurdo *et al*. 2009); gastrointestinal upset (Takahashi *et al* . 2013); gastrointestinal upset, skin redness and itching (McMurdo *et al*. 2005); mild gastrointestinal problems (Mazokopakis *et al*. 2009); abdominal discomfort (Linsenmeyer *et al*. 2004); diarrhoea, headache, and heartburn (Efros *et al*. 2010); abdominal bloating (Campbell *et al*. 2003); dyspepsia (Dohadwala *et al*. 2011); gastric pain (Bonetta and Di Pierro 2012); general weakness and lower abdominal pain (Sengupta *et al*. 2011)."

One case of hyperkalaemia was reported in a man who consumed approximately 2 L of cranberry juice daily for several days. Causality could not be determined due to concomitant medications (Thomson and Perry 2001). In another report that was likely causal, diarrhoea, hyperglycemia, and metabolic acidosis was reported in an infant given 150 mL of cranberry juice (Garcia-Calatayud *et al*. 2002).

Assessor's comment:

The following adverse events have been commonly reported in clinical trials and is included in the monograph:

Gastrointestinal disorders: nausea, vomiting, diarrhoea, constipation and dyspepsia. The frequency is not known.

Skin and subcutaneous tissue disorders: urticaria and rash. The frequency is not known.

5.4. Laboratory findings

In an 8-week study, 65 healthy female volunteers were randomized into three groups to receive placebo, 400 mg, or 1200 mg of dried cranberry juice daily. No significant changes in basic biochemical and hematological parameters were observed, including cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triacylglycerols, alanine aminotransferase, aspartate aminotransferase, D-glutamyl transferase, urea, creatinine, uric acid, and advanced oxidation protein products levels (Valentova *et al*. 2007).

Sengupta *et al* (2011) in their randomized, double blind, controlled, dose dependent clinical trial in 60 women suffering from infections of the urinary tract evaluated the hematological, and biochemical parameters in serum and urine samples collected from the low (500 mg daily) and high dose (1000 mg daily) treated groups and compared them to the untreated control group. Our detailed statistical analyses show that at the end of the 90-day follow-up period, no significant changes were observed in the hematological and serum biochemical parameters in the treatment groups. Authors suggest that the powdered cranberry product „standardised" to 1.5% proanthocyanidins is safe and nontoxic for human consumption.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

The use in children and adolescents under 18 years of age has not been documented for the authorised or registered products. Some clinical studies have been performed in children. No specific safety issue has emerged in these studies. However, the use in children and adolescents under 18 years of age is not recommended because data is not sufficient and medical advice should be sought.

5.5.2. Contraindications

Hypersensitivity to cranberry (*Vaccinium macrocarpon* Ait.) fruit.

Patients with kidney disorders experience recurrent UTI require medical supervision and the self-medication of traditional herbal medicinal products containing cranberry is contraindicated.

Concomitant use with tacrolimus and warfarin is contraindicated (see section 5.5.4).

5.5.3. Special Warnings and precautions for use

Cranberry should only be used for prevention of recurrent uncomplicated lower urinary tract infections in women, after serious conditions have been excluded by a medical doctor. Serious conditions such as pyelonephritis must be ruled out and monitored for. Furthermore, if the symptoms worsen or if complaints 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.

Long-term studies provide conflicting data regarding an association between cranberry juice or extract consumption and calcium oxalate kidney stone formation. There is no clinical evidence of increased kidney stone formation associated with cranberry consumption (Upton and Brendler 2016). Uric acid and oxalate stones may form because of acidic urine and cranberry juice's high oxalate levels. Cranberry increases urinary oxalate excretion and should be avoided in patients at risk of urolithiasis (Redmond *et al*. 2018). Therefore, a warning that cranberry concentrate has a high content of oxalate, and there may be an increased risk of stone formation in the urinary tract in patients with stone history is included in the monograph (see also section 5.5.8 'Safety in other special situations').

Health Canada (2011, 2018) gives the following Caution(s) and warning(s):

Consult a health care practitioner prior to use if you have a history of kidney stones (Gettman *et al*. 2005; Terris *et al*. 2001).

Consult a health care practitioner prior to use if you are taking blood thinners (Brinker 2010; Aston *et al*. 2006; Rindone and Murphy 2005; Grant 2004).

The use in men and pregnant women is not recommended because lower urinary tract symptoms in these populations require medical supervision.

5.5.4. Drug interactions and other forms of interaction

The American Herbal Pharmacopoeia (Upton and Brendler 2016) states that patients consuming warfarin should be aware that consumption of abnormally large quantities of cranberry could result in a bleeding event. No concern has been noted with consumption of up to 480 mL daily

There have been several case histories suggesting potentially dangerous interactions between cranberry and the blood-thinning agent warfarin.

To date, 6 cases of possible potentiation of warfarin effects with cranberry product use have been reported in the literature, including a fatality from bleeding in an elderly man taking warfarin, phenytoin, and digoxin since suffering an embolic intracerebral stroke 4 years previously (Griffiths *et al*. 2008). These events most often occurred in the elderly and are relatively rare considering the widespread consumption of cranberry juice in the elderly population. In each of the cases reported, patients had elevated INR levels associated with consumption of cranberry. Significant bleeding events occurred approximately 2 weeks after increased cranberry consumption began. In patients taking warfarin, a patient's coagulation rates are regularly checked by the INR. The target INR range for most patients on warfarin is 2.0 to 3.0 (Cushman *et al*. 2011), with values over 3.0 indicating a prolonged coagulation time and increased risk of bleeding events. The reported cases had confounding factors, such as changes in diet or a history of recent infection, which may also affect the INR level. In 3 reported cases, INR levels returned to within the therapeutic target range after discontinuation of cranberry consumption (relatively large amounts of juice or sauce) (e.g., Grant 2004; Mergenhagen and Sherman 2008; Welch and Forster 2007), providing perhaps the strongest evidence supporting that a clinically significant interaction can occur in some patients.

Following the case reports of cranberry and warfarin interactions, several human studies were completed (see Table 18). One of the studies shows a lack of interaction between cranberry and warfarin in 10 healthy volunteers (Lilja *et al*. 2007) and others in patients stable on warfarin doses (Ansell *et al*. 2009; Ansell 2011; Li *et al*. 2006; Mellen *et al*. 2010). However, the methodological quality of some of these studies has been criticized, citing too short of a study duration (usually 1 to 2 weeks) to detect a consistent clinically relevant interaction, a low patient population, or too low of a dose of warfarin to be clinically relevant (see Abdul *et al*. 2008). One study showed a significant ($P < 0.05$) 30% increase in the area under the INR–time curve when cranberry juice concentrate (equivalent to 57 g of cranberry fruit) was administered along with a high single dose of warfarin (25 mg). None of the subjects experienced major bleeding events or INR readings above 4. Two subjects developed rashes and 1 subject experienced nasal bleeding (presence of dried blood) at approximately 72 h after warfarin treatment. No significant changes in the maximum INR were observed. Cranberry did not alter S- or R-warfarin pharmacokinetics or plasma protein binding. Cranberry showed some evidence of VKORC1 (not CYP2C9) genotype-dependent interactions with warfarin. Subjects who carry the VKORC1 variant type (CT and TT alleles) were more prone to interactions with warfarin and cranberry in that cranberry significantly increased the effects of warfarin, suggesting that lower doses of warfarin are needed if taken concomitantly with a consistently characterized cranberry preparation. There was also an insignificant decrease in the activity of clotting Factor II, Factor VII, and Factor X when warfarin was co-administered with cranberry (Abdul *et al*. 2008).

In addition to the published case reports and clinical data, the Committee on Safety of Medicines (UK) as of 2003 received 7 other reports of possible warfarin-cranberry juice interactions through the formal Yellow Card reporting system of herbal practitioners. The reports suggested changes in INR or bleeding (Suvarna *et al*. 2003).

In a prospective open-label study of 10 male patients stable on warfarin, no statistical difference in prothrombin time was observed after consumption of 240 mL of pure cranberry juice twice daily for 7 days (Mellen *et al*. 2010).

In another study of patients with a stable INR on warfarin, ingestion of 240 mL cranberry juice daily for 2 weeks resulted in a mild increase in INR in 8 of 30 patients. The mean INR level was increased only on the 12th day of treatment. Cranberry juice had no effect on plasma levels of warfarin. These researchers concluded that the transient change on one study day likely would not represent a clinically relevant event and suggested that contrasting case reports may reflect chance temporal changes in INR (Ansell *et al*. 2009; Ansell 2011).

In a randomized double-blind, placebo-controlled, cross-over study, patients with atrial fibrillation on stable doses of warfarin for at least 3 months were randomized to receive either 250 mL of cranberry juice daily or placebo for 7 days, then placebo for 7 days, or vice-versa, with a washout period of 7 days. The baseline INR was 2.28 ± 0.54 for the cranberry group and 2.13 ± 0.50 for the placebo group. For all test points, the INR did not change significantly from baseline. At day 7 on cranberry juice, the INR was 2.23 ± 0.53 for the cranberry-first group and 2.16 ± 0.40 for placebo-first group. The mean differences between the cranberry and placebo groups were not statistically significant (Li *et al*. 2006).

In a study of healthy volunteers (n = 10) taking 600 mL cranberry juice and 10 mg racemic R-S-warfarin daily for 10 days, a slight decrease (7%, $P = 0.051$) in the area under the time-concentration curve of S-warfarin was observed. There were no clinically significant effects of cranberry juice on the anticoagulant effect of warfarin after 10 days of treatment, as measured by thromboplastin time (Lilja *et al*. 2007).

In an open-label, 3-treatment, randomized crossover clinical trial with 12 healthy male subjects, a single dose of 25 mg warfarin was administered alone or after 2 weeks of treatment with 1000 mg cranberry juice concentrate (equivalent to 57 g of cranberries) daily. Warfarin enantiomer concentrations, INR, platelet aggregation, and clotting factor activity were measured to assess pharmacokinetic and pharmacodynamic interactions. Cranberry extract significantly increased the area under the INR–time curve by 30% when administered with warfarin compared to warfarin alone. Maximum average INR levels were 2.6 (range 2.3–3.0) for warfarin alone and 2.8 (range 2.5–3.1) for warfarin and cranberry. Cranberry did not alter S- or R-warfarin pharmacokinetics or plasma protein binding (Abdul *et al*. 2008).

An *in vitro* and *in vivo* evaluation of 5 different commercial preparations of cranberry juice, including pure cranberry juice, pure cranberry juice concentrate, cranberry juice cocktail, and cranberry-apple juice blends, investigated the potential effects of the cranberry preparations on S-warfarin 7-hydroxylation via CYP2C9 activity. The juices were tested at different dilutions *in vitro* in human liver microsomes. One of the 5 juices significantly inhibited S-warfarin 7-hydroxylation *in vitro* in a concentration-dependent manner, indicating a potential for an *in vivo* interaction. This juice was then administered double-strength to 16 healthy volunteers before and after administration of single doses of 10 mg of S/R-warfarin. Relative to water, consumption of multiple glasses of double-strength juice had no significant impact on the total exposure of S-warfarin. However, the absorption of S-warfarin with the selected juice was slower as compared to water. The median time to the maximum plasma concentration (tmax) increased by 2 hours, and geometric mean S-warfarin maximum plasma concentration (Cmax) decreased by about 30%. Similar changes occurred with R-warfarin. No elevation in INR was reported in any of the study subjects (Ngo *et al*. 2010).

Ansell (2011), in summarizing the cranberry-warfarin case histories and formal data, noted there was "no creditable scientific evidence to link an interaction between the moderate consumption of cranberry juice and warfarin."
Ansell further reports that six of the seven interaction studies that assessed those studies in which valid and accepted pharmacodynamic and/or pharmacokinetic endpoints were used concluded that a cranberry juice-warfarin interaction is unlikely. In the seventh study, Ansell criticizes the finding based on the use of an abnormally single high dose of warfarin (25 mg) and the use of inappropriate and an unconventional area under the curve (AUC)-based pharmacodynamics parameter. Ansell does, however, note that interactions associated with large doses of cranberry cannot be ruled out and states that there are concerns with doses of up to two 8-oz glasses of juice daily. These studies examined 75 patients and healthy volunteers. A separate review of all 16 suspected reports from the UK reported to the Medicines and Healthcare products Regulatory Agency (MHRA 2003) through the Yellow Card program found that the cases were poorly documented."

In a pharmacological study of healthy volunteers, no effects of cranberry juice (200 mL daily for 10 days or 2 doses of 240 mL) were observed on the drug-metabolizing isoenzymes CYP3A4, CYP2C9, or CYP1A2 (Greenblatt *et al*. 2006; Lilja *et al*. 2007).

In an open-label, randomized, three-way crossover study, 12 healthy male volunteers received a single dose of 200 mg of the immune-suppressant cyclosporine with 240 mL of pomelo juice, cranberry juice, or water under fasting conditions. While pomelo juice significantly increased blood levels of cyclosporine, cranberry juice had no clinically significant effects on cyclosporine (Grenier *et al*. 2006).

The b-lactam antibiotics amoxicillin and cefaclor are commonly used at low doses to prevent recurrent urinary tract infections. In a crossover study, 18 healthy female volunteers received, on 4 separate occasions, a single oral test dose of amoxicillin at 500 mg and 2 g with or without 8 oz cranberry juice

cocktail. In a parallel study, 500 mg of cefaclor was administered with or without 12 oz cranberry juice cocktail. Cranberry juice cocktail delayed the absorption of both antibiotics but had no effect on the total absorption or renal clearance (Li *et al*. 2009).

A 40-year-old renal transplant patient taking cranberry extract capsules for her recurrent cystitis presented asymptomatically with low serum levels of tacrolimus. Dose increase had little effect on the level, and cessation of the cranberry extract returned levels to desired range. Cranberry extracts (1000 mg b.i.d.) are an adjunctive therapy used in the management of recurrent UTIs. Tacrolimus, an immunosuppressive agent, is metabolized intestinally by isoenzymes of the P450 cytochrome. Cranberry extracts may alter this metabolism and lead to sub-therapeutic serum levels of tacrolimus. This interaction is heretofore unreported. Cranberry extracts should be carefully monitored in allograft recipients due to interactions with serum tacrolimus levels (Dave *et al*. 2016).

Assessor's conclusion:

In spite of in vitro and animal studies with cranberry preparations showing activity on different CYP isoenzymes and Pgp inhibition, the clinical translatability is weak.

However, increased INR and increased bleeding time have been reported for patients using warfarin. There are also case reports with positive dechallenge, i.e. the INR levels returned within the normal therapeutic target range after discontinuation of intake of cranberry preparations. In the SmPC of warfarin products in EU the following warning can be found: "Cranberry juice and other cranberry products may potentiate the effect of warfarin and therefore concomitant use is contraindicated."

There is also one case report where concomitant use of cranberry juice and tacrolimus in a renal allografted patient resulted in decreased tacrolimus serum levels. The serum levels returned to normal therapeutic levels after discontinuation of the intake of cranberry juice (i.e. positive dechallenge).

The mechanisms for the possible interactions with warfarin and tacrolimus are not known.

In conclusion, the following text is included in section 4.5 of the monograph:

Cranberry juice and other cranberry products may potentiate the effect of warfarin and therefore concomitant use is contraindicated.

Decreased tacrolimus serum levels have been reported from the concomitant use of cranberry juice and tacrolimus in a renal allografted patient. The concomitant use of cranberry preparations and tacrolimus is contarindicated.

Concomitant use with tacrolimus and warfarin are included in the monograph as contraindication.

5.5.5. Fertility, pregnancy and lactation

Pregnancy

Upton and Brendler's conclusion (2016): Due to a single report of cranberry increasing the risk of bleeding in pregnant women, cranberry juice or other cranberry preparations should not be used for self-medication of urinary tract infections or suspected UTIs in pregnant women. In a single survey, spontaneous vaginal bleeding was reported in pregnant women self-medicating with cranberry, though no clinically significant risk was observed (Heitmann *et al*. 2013). A systematic review by Duguoa *et al*. (2008), reported there is no direct evidence of safety or harm to the mother or fetus as a result of consuming cranberry during pregnancy. Despite the lack of clinically significant adverse outcomes, increased bleeding in pregnancy is a serious event and should be referred to a qualified health care professional

Several reviews report that cranberry is among the most commonly used herbs in pregnancy. In pregnancy, cranberry is primarily used to prevent or treat urinary tract infection and vaginal thrush (Broussard *et al*. 2010; Forster *et al*. 2006; Holst *et al*. 2009; Kennedy *et al*. 2013; Louik *et al*. 2010; Nordeng *et al*. 2011).

No differences in obstetric or neonatal outcomes were observed in pregnant women taking **240 mL** cranberry juice cocktail, placebo, or a combination of both. In one study, pregnant women were randomized to receive research-grade **cranberry juice cocktail (27% cranberry juice**, 80 mg of PACs) **3 times daily** (n =58), cranberry juice cocktail once daily and placebo twice daily (n = 57), or placebo 3 times daily (n = 63) beginning around week 16 of pregnancy and continuing until delivery. Parameters included pre-term delivery, route of delivery (spontaneous vaginal, instrumented vaginal, or Cesarean), birth weight, 1- and 5-minute Apgar scores, and neonatal ICU admissions. Of the women enrolled, 53% of the cranberry group, 61% of the cranberry-plus-placebo group, and 68% of the placebo group completed the study. Gastrointestinal upset (nausea, vomiting, and diarrhea) was reported as a significant reason for withdrawal from the study, although information on the rate of GI upset in each of the treatment groups was not listed (Wing *et al*. 2008).

In a related pilot study on the same preparation and population, no adverse effects were reported. Pregnant women (late first trimester to early second trimester) were randomized into 3 treatment arms, each taking investigational material twice daily (morning and evening): 240 ml of cranberry juice cocktail twice daily (n = 10); 240 ml of cranberry juice cocktail in the morning, placebo in the evening (n = 9); and placebo twice daily (n =8) (Wing *et al*. 2010).

In a study on the use of herbal products during pregnancy in Norway, 600 women were interviewed within 5 days after giving birth. Of the 600, 39.7% had used at least one herbal product during pregnancy. Ginger, iron-rich herbs, echinacea, and cranberry were the most commonly used herbal products, with 6.2% of women (n = 37) having taken cranberry. A review of the birth records of women who had taken herbal products, including cranberry, indicated no adverse effects on pregnancy outcome, including birth weight, gestational length, neonatal complications, or on delivery characteristics including analgesic use during delivery, and rates of Cesarean section (Nordeng *et al*. 2011).

This same research group published a follow-up of women (n = 919 of 68,522 women surveyed) who had used cranberry while pregnant. No increased risk of adverse effect was observed regarding congenital malformations, stillbirth, neonatal death, low birth or gestational weight, preterm birth, low Apgar scores, neonatal infections, or maternal vaginal bleeding in early pregnancy. Increased vaginal bleeding in women who used cranberry to treat a UTI was observed after 17 weeks (roughly mid-pregnancy) but this did not require hospitalization and was not accompanied by any significant risk in terms of outcomes (Heitmann *et al*. 2013). Despite the lack of clinically significant adverse outcomes, increased bleeding in pregnancy is a serious event and should be referred to a qualified health care professional.

In a survey of 392 Italian women interviewed within 3 days after childbirth, 27.8% were found to have used at least one herbal product during pregnancy. The most commonly used products were chamomile (n = 48), liqorice (n = 15), fennel (n = 13), aloe (n = 11), valerian (n = 11), echinacea (n = 10), almond oil (n = 10), propolis (n = 7), and cranberry (n = 5). Birth outcomes of those who used herbs were compared with those who did not use herbs. Use of these herbs was correlated with a higher rate of infants who were small for gestational age (11.9% as compared to 5.3%), while significant differences were not observed in other outcome measures such as gestational age, birth weight, Apgar score, malformations, problems at birth, and drugs at birth (Cuzzolin *et al*. 2010). No additional analysis of the cranberry-consuming individuals was provided.

In the cohort study: *The Norwegian Mother and Child Cohort Study* wherein the data were obtained from more than 100 000 pregnant women in years: 1999-2008 informed on the use of cranberry (Heitmann *et al*. 2013). The questionnaires included weeks of pregnancy 17 and 30, and 6 months after birth, when the children were 6 months old. Exposure was classified as the use of cranberry during all pregnancy (total), its use during early pregnancy (before 17th week) and late pregnancy (at and after pregnancy week 17th). Co-medications with antibiotics were also taken into account. Outcome variables were based on ICD-10 code classification. They were: malformations, stillbirth/neonatal death, low birth weight (<2500g) small for gestational age, pretherm birth, Apgar score<7 at 5 minutes and neonatal infections. The questionnaires also provided information on maternal vaginal bleeding. Maternal UTI were also included. In statistical analyses Pearson's chi-square test, Fisher's exact test, univariate and multivariate logistic regression were used with p-values of <0.05. In total over 68 000 women was included in the study and 919 (1,3%) had used cranberry during pregnancy. Of the women who used cranberry during pregnancy, 61.6% had used it in early pregnancy, and 60.3% had experienced UTI. There was a correlation between cranberry use in late pregnancy and vaginal bleeding after the 17th week of pregnancy, however, subsequent subanalysis did not present significant risk. Nevertheless, a non-significant trend was found during late pregnancy after the 17th week between cranberry and vaginal bleeding (more than spotting). This phenomenon should be confirmed or excluded in further studies.

Lactation

No data are available. Based on its widespread consumption as a food, no negative effects with normal to therapeutic doses are to be expected.

Fertility

No studies on the effects on fertility have been performed.

Assessor's comment:

No fertility data 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

No data available.

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

Gettman *et al* 2005 evaluated the effect of cranberry juice on urinary stone risk factors.

A total of 12 normal subjects and 12 calcium oxalate stone formers underwent 2, 7-day phases of study in random order while on a controlled metabolic diet.

Subjects ingested 1 l of cranberry juice (CBJ) daily in 1 phase and 1 l of deionized water in the other phase. On the last 2 days of each phase 2, 24-hour urine collections and blood samples were obtained for stone risk factors and serum chemistries.

No significant differences were found between normal subjects and stone formers in response to CBJ and, therefore, the groups were combined. CBJ significantly increased urinary calcium (from 154 to 177 mg per day, p=0.0008) and urinary oxalate (from 26.4 to 29.2 mg per day, p =0.04), thereby increasing urinary saturation of calcium oxalate by 18%. Urinary citrate was unchanged and urinary magnesium increased slightly. Urinary pH decreased (from 5.97 to 5.67, p=0.0005), and urinary ammonium, titratable acidity and net acid excretion increased during CBJ ingestion. Urinary uric acid decreased (from 544 to 442 mg per day, p <0.0001) as did serum uric acid. Thus, the urinary saturation of brushite and monosodium urate was reduced by CBJ but the amount of undissociated uric acid increased. Authors concluded that CBJ exerts a mixed effect on urinary stone forming propensity. It reduces urinary pH likely by providing an acid load and decreases urinary uric acid perhaps by retarding urate synthesis. Overall CBJ increases the risk of calcium oxalate and uric acid stone formation but decreases the risk of brushite stones.

Assessor's comment:

Cranberry concentrate has a high content of oxalate, and there may be an increased risk of stone formation in the urinary tract in patients with stone history. A warning is included in the monograph.

Patients with kidney disorders experience recurrent UTI require medical supervision and the self-medication of traditional herbal medicinal products containing cranberry is contraindicated.

5.6. Overall conclusions on clinical safety

In clinical trials, most adverse effects have been related to the gastrointestinal tract (e.g., nausea, vomiting, diarrhoea, constipation and dyspepsía) and are transient.

Urticaria and skin rash have also been reported.

Cranberry juice and other cranberry products may potentiate the effect of warfarin and therefore concomitant use is contraindicated. Furthermore, decreased tacrolimus serum levels have been reported from the concomitant use of cranberry juice and tacrolimus in a renal allografted patient. The concomitant use of cranberry preparations and tacrolimus is contraindicated.

Cranberry should only be used for prevention or in the relief of symptoms of recurrent uncomplicated lower urinary tract infections in women, after serious conditions have been excluded by a medical doctor. If the symptoms worsen or if complaints such as fever, dysuria, spasms, or blood in urine occur during the use cranberry medicinal product, a doctor or a qualified health care practitioner should be consulted.

Patients with kidney disorders experience recurrent UTI require medical supervision and the self-medication of traditional herbal medicinal products containing cranberry is contraindicated.

The use in children and adolescents under 18 years of age is not recommended because data is not sufficient and medical advice should be sought.

The use in men and pregnant women is not recommended because lower urinary tract symptoms in these populations require medical supervision. Safety during pregnancy and lactation has not been established.

Cranberry concentrate has a high content of oxalate, and there may be an increased risk of stone formation in the urinary tract in patients with stone history.

6. Overall conclusions (benefit-risk assessment)

A number of randomised, placebo-controlled, and double-blind clinical trials have been conducted for the evaluation of cranberry juice in prevention of recurrent UTI. However, the studies report both positive and negative outcome and a coherence of scientific assessment is lacking. There is also inconsistency between the studies regarding the criteria that were used to diagnose a UTI. It should be a clear definition of "breakthrough" cases in the studies and microbiologically confirmed UTI is considered the highest evidence of a breakthrough.

Additional studies found in the literature show significant limitations, including insufficiently described cranberry preparation and poor study design e.g. small numbers of subjects, the lack of a control group, or not double blind, short duration of study, as well as high drop-out rate in some of the studies, that considerably limit their value as a source of evidence to substantiate efficacy in prevention of recurrent UTI.

Overall, the requirements for well-established medicinal use according to Article 10a of Directive 2001/83/EC is considered not fulfilled.

Fifteen years of use of Cranberry juice in the European Union is considered demonstrated based literature data and on the fact that a medicinal product with concentrated juice has been authorised in Denmark and Iceland in 1996. Thirty years of medical use for prevention and relief of symptoms of recurrent UTI is documented in several sources.Taking into account all of these historical data on the traditional use of cranberry from North America, it can be concluded, that the 30 years of requirement in medicinal use is fulfilled for the Cranberry juice.

In conclusion, the following herbal preparation fulfil the criteria for traditional medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA:

Expressed juice from the fresh fruit - (DER 1: 0.6-0.9)

Indication 1)

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.

Indication 2)

Traditional herbal medicinal product used for prevention of recurrent uncomplicated lower urinary tract infections in women, after serious conditions have been excluded by a medical doctor.

The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

In clinical trials, most adverse effects have been related to the gastrointestinal tract (e.g., nausea, vomiting, diarrhoea, constipation and dyspepsía) and are transient. Urticaria and skin rash have also been reported.

Cranberry juice and other cranberry products may potentiate the effect of warfarin and therefore concomitant use is contraindicated. Furthermore, decreased tacrolimus serum levels have been

reported from the concomitant use of cranberry juice and tacrolimus in a renal allografted patient. The concomitant use of cranberry preparations and tacrolimus is contraindicated.

Cranberry should only be used for prevention or in the relief of symptoms of recurrent uncomplicated lower urinary tract infections in women, after serious conditions have been excluded by a medical doctor. If the symptoms worsen or if complaints such as fever, dysuria, spasms, or blood in urine occur during the use cranberry medicinal product, a doctor or a qualified health care practitioner should be consulted.

Patients with kidney disorders experience recurrent UTI require medical supervision and the self-medication of traditional herbal medicinal products containing cranberry is contraindicated.

The use in children and adolescents under 18 years of age is not recommended because data is not sufficient and medical advice should be sought.

The use in men and pregnant women is not recommended because lower urinary tract symptoms in these populations require medical supervision. Safety during pregnancy and lactation has not been established. The use during pregnancy and lactation is not recommended.

Cranberry concentrate has a high content of oxalate, and there may be an increased risk of stone formation in the urinary tract in patients with stone history.

Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed on the herbal preparation in the monograph.

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

Typical analytical markers are proanthocyanidins.

<Annex><Annexes>

List of references