



20 November 2012
EMA/HMPC/136022/2010
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Cucurbita pepo* L., semen

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

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Cucurbita pepo</i> L., semen
Herbal preparation(s)	a) Comminuted herbal substance b) Soft extract (DER 15-25:1), extraction solvent ethanol 92% m/m c) Dry extract (DER 15-30:1) extraction solvent ethanol 60% v/v d) Fatty oil
Pharmaceutical forms	Herbal substance or herbal preparations in solid dosage form for oral use
Rapporteur	Ewa Widy-Tyszkiewicz
Assessor(s)	Ewa Widy-Tyszkiewicz Irena Matławska Wiesława Bylka



Table of contents

Table of contents	2
1. Introduction	3
1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof ..	3
1.2. Information about products on the market in the Member States	10
Regulatory status overview	10
1.3. Search and assessment methodology	14
2. Historical data on medicinal use	14
2.1. Information on period of medicinal use in the Community	14
2.2. Information on traditional/current indications and specified substances/preparations ..	15
2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications.....	17
3. Non-Clinical Data	19
3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	19
3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	28
3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof	29
3.4. Overall conclusions on non-clinical data	30
4. Clinical Data	30
4.1. Clinical Pharmacology	30
4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	30
4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents	31
4.2. Clinical Efficacy	31
4.2.1. Dose response studies.....	31
4.2.2. Clinical studies (case studies and clinical trials)	31
4.2.3. Clinical studies in special populations (e.g. elderly and children).....	41
4.3. Overall conclusions on clinical pharmacology and efficacy	41
5. Clinical Safety/Pharmacovigilance	41
5.1. Overview of toxicological/safety data from clinical trials in humans.....	41
5.2. Patient exposure	41
5.3. Adverse events and serious adverse events and deaths	42
5.4. Laboratory findings.....	42
5.5. Safety in special populations and situations	42
5.6. Overall conclusions on clinical safety.....	43
6. Overall conclusions	43
Annex	44
List of references	44

1. Introduction

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

- Herbal substance(s)

The herbal substance consists of the whole, ripe and dried seeds.

Cucurbita pepo L. (pumpkin) belongs to the melon family Cucurbitaceae which comprises approximately 95 genera and 950-980 species (Schaefer and Renner 2011).

Cucurbita pepo is indigenous to warm and temperate regions of Central and North America and is cultivated there. It also exists in wild form in Europe and Asia. The origin is uncertain. The common ancestor of all the current *Cucurbita pepo* varieties originates probably from Mexico as confirmed by archeological findings (Andres 2003). The herbal substance (whole, dried, ripe seed) is mentioned in several well-known handbooks, such as Madaus (1938), Bradley (2006), Martindale (2007), Wichtl (2004), Gruenwald *et al.* (2000; 2004), German Commission E Monograph (1991), Duke's Handbook of Medicinal Herbs (Duke 2000), WHO Monographs (2009) and ESCOP Monographs (2009).

The seeds and oil from pumpkin seeds have been used for many years for the relief of difficulties associated with an enlarged prostate gland and micturition problems related to overactive bladder. The pumpkin seeds yield approximately 50% fatty oil, (mostly linoleic and oleic acid and tocopherol), but the putative active constituents are Δ^7 -sterols (avenasterol, spinasterol) and Δ^5 -sterols (sitosterol, stigmasterol).

At the moment, there is no published monograph in the European Pharmacopoeia. The definition in the German Pharmacopoeia is: "whole, dry and ripe seeds of *Cucurbita pepo* L. and/or other diverse cultivated varieties" (Deutsches Arzneibuch 1999). Typically, the variety *C. pepo* L. convar. *citrullina* I. Greb. var. *styriaca* I. Greb. is used for pharmaceutical purposes because of the thin testa and the soft taste of the seeds. In many countries, seeds of plants of the Cucurbitaceae family are popular for light meals. They are categorised to *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita mixta* and *Cucurbita moschata* according to the pattern and the structure of their branches.

Constituents of the pumpkin seeds

In old literature, the data on the composition of pumpkin seeds are rather inconsistent, because in many cases details of the variety and cultivar, or even the full botanical name, are not given (Bradley 2006; Bombardelli and Morazzoni 1997). The content of amino acids, fatty acids and minerals may vary considerably, depending on different conditions (Glew *et al.* 2006). Such differences may be caused by variations in cultivar or origin (Tsaknis *et al.* 1997).

- **Fatty oil**

The fatty oil is obtained from comminuted seeds which are roasted immediately before pressing. The physical-chemical characteristics of the oil and its content of fatty acids, tocopherols, carotenoids, chlorophyll pigments, squalene and sterols are described in the literature (Bombardelli and Morazzoni 1997; Fruhwirth and Hermetter 2007; Fruhwirth and Hermetter 2008; Fruhwirth *et al.* 2003).

The fatty oil content of pumpkin seed is about 50% (45-60%) (Murkovic *et al.* 1996b; Tsaknis *et al.* 1997; Wallner-Liebmann 2011).

Pumpkin (*Cucurbita pepo* L.) seed oil is dark green and has a high content of free fatty acids (Murkovic *et al.* 1996a; Tsaknis *et al.* 1997). Due to its colour and the foam formation, the oil is not used for cooking (Murkovic *et al.* 2004). When obtained by pressing, the oil has a dark red to green colour (due to content of carotenoids and chlorophylls), a red fluorescence and a nutty taste (Vogel 1978; Sauter *et al.* 1985; Murkovic *et al.* 1996a; Tsaknis *et al.* 1997; Kreft *et al.* 2009).

Many factors influence the chemical composition of pumpkin seeds grown in different conditions. Hence the differences presented in many publications.

The composition of fatty acids varies depending on several factors: variety of areas in which the plants are grown, climate, growth environment, state of ripeness. The variability in the oil content is very high, resulting from a broad genetic diversity, harvesting conditions, storage period, storage environment and processing procedures (Murkovic *et al.* 1996a; Alfawaz 2004; Ardabili *et al.* 2011).

The glyceride fraction content ranging from 73.1% to 80.7 % unsaturated fatty acids, mainly linoleic (C18:2; 39.8 -64%) and oleic (C18:1; 20-38%) acids, and 19% saturated fatty acids, mainly palmitic (C16:0; ca. 13%) and stearic acids (C18:0; ca. 6%) (Ardabili *et al.* 2011, Andrikopoulos *et al.* 2004; Murkovic *et al.* 1996a; Stevenson *et al.* 2007; Vogel 1978; Tsaknis *et al.* 1997; Bombardelli and Morazzoni 1997).

Several studies have reported similar proportions of total fatty acids or free fatty acids (Al-Khalifa 1996; Bravi *et al.* 2006; El-Adawy and Taha 2001; Glew *et al.* 2006, Haiyan *et al.* 2007; Hethelyi *et al.* 1989; Küsmenoğlu 1996; Parry *et al.* 2008; Pranabendu *et al.* 2009). However, Zdunczyk *et al.* (1999) presented different unusual proportions of fatty acids in a pumpkin seed cake: 50.4% oleic and 29.9% linoleic acid.

The fatty acid composition of the crude lipid fraction of seeds of *Cucurbita* spp. of Nigerian origin with four fatty acids accounting for > 97% of the fatty acid total: palmitic acid (C 16:0; 13.0%), stearic acid (C 18:0; 7.9%), oleic acid (C 18: 1 n-9; 45.4%) and the essential fatty acid linoleic acid (C 18:2n-6; 31%). On a percentage basis, oleic acid was the predominant fatty acid. Whereas linoleic acid accounts for nearly one-third of the total fatty acid in pumpkin seeds, only trace amounts of α - and γ -linolenic acids were found (Glew *et al.* 2006).

Table 1. Fatty acid composition of the pumpkin seed (*Cucurbita pepo* subsp. *pepo* var. *styriaca*) oil.^a (Ardabili *et al.* 2011)

Fatty acid	Content (%)
Palmitic (C _{16:0})	10.68 ± 0.42
Palmitoleic (C _{16:1})	0.58 ± 0.14
Stearic (C _{16:1})	8.67 ± 0.27
Oleic (C _{18:1})	38.42 ± 0.37
Linoleic (C _{18:2})	39.84 ± 0.08
Linolenic (C _{18:3})	0.68 0.14
Gadoleic (C _{20:1})	1.14 ± 0.00
Total saturated fatty acids	19.35 ± 0.16
Total unsaturated fatty acids	80.65 ± 0.16

^a Means of duplicate determinations

The content of selected fatty acids composition in commercial pumpkin seed oil purchased in local marked (Italy) were determined: palmitic 12.0 mg/100 mg; stearic 6.6 mg/100 mg; oleic 28.1 mg/100 mg; linoleic 36.1 mg/100 mg; linolenic 1.9 mg/100 mg (Bravi *et al.* 2006).

Older data show that in different varieties used for oil production, palmitic acid occurs in the range of 10.3% to 11.7%, stearic acid 4.1 % to 5.4%, oleic acid 30.5% to 40.8% and linoleic acid 42.1% to 51.5% (Murkovic *et al.* 1996a).

The dominant fatty acids found in the seeds of the 100 breeding lines of *Cucurbita pepo* L. convar. *citrullina* var. *styriaca* with dark green naked seeds and long shoots are: palmitic (C16:0; 9.5-14.5%), stearic (C18:0; 3.1-7.4%), oleic (C18:1; 21.0-46.9%) and linoleic (C18:2; 35.6-60.8%) acids. The content of these four fatty acids ranges from 98.1 to 98.7% and the other being found at level below 0.5% (Murkovic *et al.* 1996a).

Those pumpkins which need a long time for ripening and which are harvested very late show a higher content of linoleic acid. This may be a result of the colder climate later in the year which normally leads to oil with a higher content of polyunsaturated fatty acids and probably reflects the higher activity of microsomal oleoylphosphatidylcholine desaturase at lower temperature (Murkovic *et al.* 1996a).

Content of unsaponifiable fraction

Sterols, up to 0.5% of the oil (55-60% of the unsaponifiable fraction), predominantly Δ^7 -sterols (Δ^7 or delta-7, signifies a double bond between C-7 and C-8). Much smaller amounts of Δ^5 - and Δ^8 -sterols are also present (Bastic *et al.* 1977; Garg and Nes 1986).

Four Δ^7 -sterols account for 75-88% of the total sterols (Tsaknis *et al.* 1997; Bastic *et al.* 1977; Akihisa *et al.* 1986, 1987) and 40-50% of the unsaponifiable part of the oil (Sauter *et al.* 1985): $\Delta^{7,25}$ -stigmastadienol (stigmasta - 7,25-dien -3 β -ol), $\Delta^{7,22}$ -stigmastadienol = α -spinasterol (stigmasta - 7,22-dien-3 β -ol), $\Delta^{7,22,25}$ -stigmastatrienol (stigmasta - 7, 22, 25-trien-3 β - ol), Δ^7 -ergosterol (27-methylcholest-7-en-3 β -ol) and $\Delta^{7,24,28}$ -stigmastadienol = Δ^7 -avenasterol (stigmasta-7, 24 (28) - dien-3 β -ol) (Sauter *et al.* 1985; Akihisa *et al.* 1986; Tsaknis *et al.* 1997; Bastic *et al.* 1977; Bombardelli and Morazzoni 1997).

In the literature, different authors use varying chemical terminology for the names of sterols; for example: $\Delta^{7,25}$ -stigmastadienol (Tsaknis *et al.* 1997) is also described as $\Delta^{7,25,27}$ -stigmastadien-3 β -ol (Bombardelli and Morazzoni 1997); 5 α -stigmasta-7,25-dien-3 β -ol (Sauter *et al.* 1985) and 24 β -ethyl - 5 α -cholesta-7,25 (27)-dien-3 β -ol (Akihisa *et al.* 1986).

The sterol fraction of pumpkin seed oil of *C. pepo* and *C. maxima* beside of predominated compounds: $\Delta^{7,22,25}$ -stigmastatrien- 3 β - ol, α -spinasterol, $\Delta^{7,25}$ -stigmastadienol and Δ^7 -avenasterol, contains also: stigmasterol (stigmasta-5,22-dien-3 β -ol), 24-methylcholest-7-enol and Δ^7 -stigmastenol (stigmasta-7-en-3 β - ol), and trace amounts of cholesterol (cholest-5-en - 3 β - ol), brassicasterol (ergosta-2,22-dien-3 β -ol), campesterol (campest-5-en-3 β -ol), sitostanol (stigmastan-3 β -ol), Δ^5 -avenasterol (stigma-5,24(28)-dien-3 β -ol), erythrodiol, and uvaol (Tsaknis *et al.* 1997).

In the seeds of *C. pepo* L. convar. *citrullina* Greb. var. *styriaca* Greb. 24-ethyl- Δ^7 -steryl glucosides were identified as: 3-O-(β -D- glucopyranosyl) - 24 β -ethyl-5 α -cholesta-7,25 (27)-dien-3 β -ol, 3-O-(β -D- glucopyranosyl) -24 β -ethyl-5 α -cholesta-7, trans-22, 25 (27)-trien-3 β -ol, 3-O-(β -D- glucopyranosyl) 24 α -ethylsterol and spinasterol (Rauwald *et al.* 1985).

The predominance of Δ^7 -sterols in pumpkin seed oil is in contrast to the sterol fractions of most seed oils, in which Δ^5 -sterols (30-60%) are usually predominant (Sauter *et al.* 1985). The free 24-ethyl- Δ^7 -sterols are typical for the seeds of some of Cucurbitaceae, whereas reports of glucosylated Δ^7 -sterols

are very rare, possibly due to the difficult chromatographic separation of this type of glycosides (Rauwald *et al.* 1985; Sauter *et al.* 1985).

Squalene: Squalene (39-46%) is the characteristic constituent of the unsaponifiable fraction of the oil seeds. It can be used as a marker for the differentiation of oils obtained from other seeds (Sauter *et al.* 1985; Bombardelli and Morazzoni 1997).

Triterpenoids: including 0.08-0.2% of multiflorane p-aminobenzoates (7-epi zucchini factor A and debenzoyl zucchini factor B) (Appendino *et al.* 1999; 2000).

Fruits of cultivated pumpkins have been cultured to be free of cucurbitacins and are assumed to contain a suppressor gene or a mutation responsible for absence of cucurbitacins. However, back-mutations occur randomly which may lead to plants with toxic and bitter fruits. Therefore the presence of cucurbitacins in seeds cannot be excluded (EFSA Compendium 2009; Schmandke 2008; Wang *et al.* 2008).

- **Sesquiterpenoids**

The following monocyclic sesquiterpenoids have also been isolated: oxycerotic acid, (+)-abscisic acid, (+) - 2-trans-abscisic acid, (+) - dehydrovomifoliol and (+) vomifoliol (Bombardelli and Morazzoni 1997; Bradley 2006).

- **Tocopherols**

ca. 360-540 mg/kg of oil, comprise β - and γ - (but not α -) tocopherols (Vogel 1978). Pumpkin seeds were found to have the greatest content of tocopherols (16 mg/100 g) with β - and γ -tocopherol being predominant over α -tocopherol (Ryan *et al.* 2007). However, only α -tocopherol was detected in pumpkin seed crude oil, at a level of 126 mg/kg, with reduction to 78 mg/kg after purification of the oil (Tsaknis *et al.* 1997). That finding, even with significantly greater reductions of up to 33%, was confirmed by Lazos (1992).

Other report showed an average of 338 mg/kg of β - and γ -tocopherols only, while α - and δ -tocopherols were not found or higher tocopherol content averaging 437 mg/kg, without any reference to tocopherol form (Tsaknis *et al.* 1997).

The content of vitamin E, especially γ -tocopherol, is very high (Sauter *et al.* 1985). According to Murkovic *et al.* (1996b), the γ -tocopherol content is about 5-10 higher than α -tocopherol. The concentration of γ -tocopherol, which is the dominant tocopherol in pumpkin oil seed, from the 100 lines of *Cucurbita pepo* L. convar. *citrullina* var. *styriaca* with dark green naked seeds and long shoots, is in the range of 41 to 620 mg/kg (Murkovic *et al.* 1996b). The range of concentrations of α -tocopherol varies from 0 to 91 mg/kg (Murkovic *et al.* 1996b).

The level of both β - and δ -tocopherol is very low, but sporadically can reach 16 mg/kg and 49 mg/kg respectively. β -tocopherol was below the detection limit in 90 out of 100 samples and δ -tocopherol in 82 (Murkovic *et al.* 1996b). After roasting of pumpkin seeds, the total tocopherol loss was 36%, the highest was for β -tocopherol at 50%; for α -tocopherol it was 41% and for γ -tocopherol it was 36% (Gemrot *et al.* 2006).

- **Carotenoids**

15 ppm (Vogel 1978), mainly lutein (50%) and β -carotene (10-12%) with smaller amounts of cryptoxanthin and various other carotenoids (Vogel 1978; Matus *et al.* 1993; Bombardelli and Morazzoni 1997; Murkovic *et al.* 2002; Azevedo-Meleiro and Rodriguez-Amaya 2007).

- **Minerals**

Particularly phosphorus, potassium, magnesium, calcium, iron, zinc and trace elements (Mansour *et al.* 1993). Selenium is of particular importance as its content ranges between 0.08 and 0.4 µg/g, one of the highest values found in plants (Bombardelli and Morazzoni 1997; Kreft *et al.* 2002). Other sources report even higher amount of selenium: 1.29 µg/g (Glew *et al.* 2006). The mean estimated maximum intake was found to be 0.4 mg/day and the safe upper level was estimated as 0.45 mg total selenium/day for daily consumption over a lifetime (EFSA Scientific Opinion 2009).

The pumpkin seeds contained relatively large amounts of potassium (5,790 µg/g dry weight) and chromium (approximately 3 µg/g dry weight). However, the sodium content of pumpkin seeds was low (6.9 µg/g dry weight). Pumpkin seeds from Niger contained relatively large amounts of magnesium (5,690); zinc (113); copper (15.4); molybdenum (0.805) and another minerals: phosphorus (15,700); calcium (346); iron (106); manganese (49.3); aluminum (9.21); barium (1.16); cobalt (0.29); strontium (1.83); nickel (0.53); arsenic (0.45) (in µg/g dry weight). Noteworthy are the low amounts of calcium in the seeds (Glew *et al.* 2006).

According to Suphakarn *et al.* (1987), 100 g roasted pumpkin seeds contain: calcium 25.94 mg, phosphorus 955.81 mg, and iron 8.06 mg.

Pumpkin seed of *Cucurbita pepo* Kakai 35 from Hungary contained: phosphorus 17.8 g/kg; potassium 13.7 g/kg; magnesium 5.7 g/kg; calcium 1.6 g/kg; iron 0.2 g/kg; sodium 0.3 g/kg; copper 0.02 g/kg; zinc 0.19 g/kg; manganese 0.08 g/kg (Mansour *et al.* 1993).

- **Proteins and amino acids**

Proteins are abundantly present in the seeds (31% - 51%) (Bombardelli and Morazzoni 1997; Bradley 2006; Glew *et al.* 2006).

Fractionation of the proteins of *Cucurbita pepo* seeds revealed considerable differences in solubility of the total salt-soluble protein fraction. The major component had crystalline form and solubility at 40% of (NH₄)₂SO₄ saturation and the minor component had solubility maximum at 60% saturation (Alekseeva 1965).

Cucurbitae seed globulins have nutritive value as they were found in laboratory rats in old experiment (Hubbell *et al.* 1942). They have a high content of aspartic acid, glutamic acid and arginine and low lysine content (Kimmel and Smith 1958). The following amino acids were released from proteins by HCl hydrolysis: alanine (23.4); arginine (93.2); aspartic acid (52.8); cysteine (6.73); glutamic acid (104); glycine (28.3); histidine (13.8); isoleucine (23.0); leucine (40.9); lysine (22.0); methionine (12.4); phenylalanine (31.4); proline (20.2); serine (31.7); threonine (18.4); tryptophan (15.3); tyrosine (22.1) and valine (28.2) (in mg/g dry weight) (Glew *et al.* 2006).

According to Kim *et al.* (2012) the raw pumpkin seed (*Cucurbita pepo*) contain high levels of most essential amino acids (Table 2).

Table 2. Amino acids concentrations in *Cucurbita pepo* raw seeds (Kim *et al.* 2012)

Amino acid concentrations	Mg/kg raw weight
	Mean \pm SD
Alanine	17.76 \pm 0.03
Arginine	63.99 \pm 0.88
Aspartic acid	29.95 \pm 0.25
Glutamic acid	60.26 0.04
Glycine	18.70 \pm 0.36
Histidine	18.37 \pm 0.08
Isoleucine	13.96 \pm 0.74
Leucine	24.14 \pm 0.96
Lysine	13.14 \pm 0.48
Methionine	4.20 \pm 0.37
Phenylalanine	15.52 \pm 0.53
Proline	11.98 \pm 0.37
Serine	14.99 \pm 0.21
Threonine	7.56 \pm 0.07
Tyrosine	8.18 \pm 0.01
Valine	17.43 \pm 0.69

Rare amino acids have also been found in *Cucurbita pepo* seeds: m-carboxyphenylalanine, β -pyrazolalanine, γ -aminobutyric acid, ethyl-asparagine and citrulline. Particular attention has been paid to cucurbitine (3-amino-3-carboxy-pyrrolidine) for its anthelmintic properties isolated from *Cucurbita pepo* (0.18-0.66%) and *Cucurbita moschata* Duch., (0.4-0.84%) seeds. Cucurbitine content varied even within the same species (Blagrove and Lilley 1980; Bombardelli and Morazzoni 1997; Bradley 2006; Bruneton 1995; Huang 1998; Mihranian and Abou-Chaar 1968; Rybałowski 1966).

Moschatin, a novel type I ribosome-inactivating protein (RIP) from mature seeds of pumpkin (*Cucurbita moschata*) and from sacrocarp of *Cucurbita pepo* and *C. moschata* were found (Barbieri *et al.* 2006).

The cucurmoschin, a peptide with a molecular mass of 8 kDa and rich in arginine, glutamate and glycine residues was isolated from the seeds of *Cucurbita moschata* (Wang and Ng 2003).

The abumin ribonuclease (RNase) (2S albumin) was purified from pumpkin seeds (*Cucurbita* sp.) (Fang *et al.* 2010). From the seeds of *C. pepo* and other members of the Cucurbitaceae, some low molecular weight (MW) trypsin, Hageman factor, human leucocyte elastase and cathepsin G inhibitors have been isolated (Leluk 2000; Otlewski *et al.* 1984; Otlewski and Wilusz 1985; Otlewski and Krowarsch 1996). The compounds are polypeptides composed of approximately 30 amino acids, with three disulphide bridges and fold in a novel knotting structure. Inhibitors of trypsin and activated Hageman factor with low MW \approx 3,000-4,000 were isolated independently by Polanowski *et al.* (1980; 1987) and Hojima *et al.* (1982) from *Cucurbita pepo* and *Cucurbita maxima* seeds.

Peponin, a single-chain RIP with a MW of about 30 kDa was isolated from pumpkin seed by bioactivity-guided fractionation (Gerhäuser *et al.* 1993).

- **Carbohydrates**

The carbohydrate content is between 6% and 10% (Sauter *et al.* 1985; Bombardelli and Morazzoni 1997). The dehulled and defatted pumpkin seeds of *Cucurbita pepo* Kakai 35, commercially cultivated throughout Hungary, contain 91.3 g/kg of total carbohydrate, among them were identified: sucrose (17.9 g/kg); raffinose (4.1 g/kg); stachyose (8.1 g/kg); fructose was not detected (Mansour *et al.* 1993).

- **Vitamins**

In pumpkin seed of *Cucurbita pepo* Kakai 35 are present B group vitamins: thiamine 6.89; riboflavin 2.47; niacin 61.43; pyridoxine 4.92; pantothenic acid 4.95 (mg/kg) (Mansour *et al.* 1993).

A proximate analysis of 100 g roasted pumpkin seeds revealed: vitamin A 3.32 µg, thiamin 0.28 mg, riboflavin 0.05 mg, vitamin C 30.38 mg, niacin 0.95 mg (Suphakarn *et al.* 1987).

- **Phenolic glycosides**

Eight new phenolic glycosides, cucurbitosides F-M, were isolated from the seeds of *Cucurbita pepo*. Their structures were elucidated as 4 - (2 - hydroxyethyl) phenyl 5- O - (2 - S - 2- methylbutyryl) - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, 4 - (2 - hydroxyethyl) phenyl 5- O - (3 - methylbutyryl) - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, 4 - (2 - hydroxyethyl) phenyl 5- O - nicotinyl - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, 4 - (2 - hydroxyethyl) phenyl 5- O - (4 - aminobenzoyl) - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, 4 - (2 - hydroxyethyl) - 2 - methoxyphenyl 5- O - (2 - S - 2 - methylbutyryl) - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, 4 - (hydroxymethyl) phenyl 5- O - (2 - S - 2 - methylbutyryl) - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, 4 - (hydroxymethyl) phenyl 5- O - nicotinyl - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, and 4 - (hydroxymethyl) phenyl 5- O - (4 - aminobenzoyl) - β - D - apiofuranosyl (1→2) - β - D - glucopyranoside, on the basis of various spectroscopic analyses and analyses of hydrolysis product.

The structures are closely related to cucurbitosides A-E from the seeds of *C. moschata*. All these compounds contain the 5- O - acyl - β - D - apiofuranosyl (1→2) - β - D - glucopyranose moiety in the structures, which appears to be a characteristic constituent of pumpkin seeds (Li *et al.* 2005).

- **Lignans**

Secoisolariciresinol and lariciresinol were identified in pumpkin seeds (Sicilia *et al.* 2003).

- **Other compounds**

Other substances present in ripe seeds include cucurbitol and polyalcohols present as esters of phosphoric acid (Bombardelli and Morazzoni 1997), small amounts of gibberellins, a kaurenolide, cucurbic acid and other acids and esters (MacMillan 1997).

Seeds of pumpkin (*Cucurbita pepo* L.) contain three chromatographically distinguishable cytokinins extractable by ethanol and n-butanol. One of these cytokinins are similar to those of zeatin, whereas other two of them do not match with those of any of the known natural cytokinins (Gupta Geeta and Maheswari 1970).

- Herbal preparation(s)

Comminuted herbal substance

Soft extract (DER 15-25:1), extraction solvent ethanol 92% m/m

Dry extract (DER 15-30:1), extraction solvent ethanol 60% v/v

Fatty oil

- 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. Information about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Also in combination products
Belgium	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Denmark	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Estonia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
France	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Germany	<input checked="" type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Also in combination products
Greece	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
Hungary	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Also in combination product
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Italy	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Food supplements
Latvia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Lithuania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	

Member State	Regulatory Status				Comments
Luxemburg	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Malta	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Food supplements
Norway	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Poland	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Food supplements
Portugal	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
Romania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Slovak Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
Slovenia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Spain	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised or registered herbal medicinal products
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
United Kingdom	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

Table 3. Products on the market

Active substance	Indication	Posology	Period of medicinal use
1 capsule, soft: Cucurbitae semen soft extract (15-25:1), extraction solvent ethanol 92% m/m	For relief of lower urinary tract symptoms related to benign prostatic hyperplasia (Alken stages I to II or Vahlensieck stages II to III).	Adults: 2 times daily 1 capsule containing 500 mg dry extract	At least since 1976 (WEU, authorised, Germany)
1 capsule, soft: Cucurbitae* oleum 1000 mg	Traditionally used to strengthen bladder function	Adults and adolescents: 3-4 capsules daily	at least since 1976 (TU, authorised**, Germany)
1 capsule, hard: Cucurbitae* semen dry extract (15-30:1), extraction solvent ethanol 60% v/v 105 mg		Adults and adolescents: 1 capsule 3 times daily	at least since 1976 (TU, authorised**, Germany)

Active substance	Indication	Posology	Period of medicinal use
Granules: 100 g granules contain 66 g Cucurbitae* semen		Adults: 8-23 g granules daily (corresp. 5-15 g Cucurbitae semen) Adolescents: 3-6 g granules daily	at least since 1976 (TU, authorised**, Germany)
Herbal substance (Cucurbitae* semen)		Adults and adolescents: 5-15 g daily divided in 2 doses (one in the morning and one in the evening)	at least since 1976 (TU, authorised**, Germany)
1 capsule, soft: Cucurbitae* oleum 583 mg		Adults and adolescents: 2 capsules 3 times daily Daily dose : 3498 mg of Cucurbitae oleum	at least since 1976 (TU, authorised**, Germany)
1 capsule, soft: Cucurbitae* oleum 583 mg		Adults and adolescents: 2 capsules 3 times daily Daily dose : 3498 mg of Cucurbitae oleum	at least since 1976 (TU, authorised**, Germany)
1 film-coated tablet: Cucurbitae* semen dry extract (15-30:1), extraction solvent ethanol 60% v/v 152 mg		Adults and adolescents: 1 tablet 2 times daily	at least since 1976 (TU, authorised**, Germany)
1 capsule, soft: Cucurbitae peponis oleum 300 mg	For decreasing symptoms associated with an enlarged prostate (irritable bladder with urgency, night urgency and urge incontinence), prevention and supportive treatment of cardiovascular disease.	2 capsules 3 times daily	1988 (registered, Hungary)
1 capsule, soft: Cucurbitae peponis oleum virginum 300 mg	Traditionally in troubles with urination caused by	2 capsules 2 times daily before meals, during	1990 (authorised, Poland)

Active substance	Indication	Posology	Period of medicinal use
	benign prostatic hyperplasia It is recommended to regularly perform urological examinations	4 weeks	

* *Cucurbita pepo* L. convar. *citrullina* I. Greb. var. *styriaca* I. Greb.

**For the sake of completeness, all preparations for which marketing authorisations for traditional use have been granted (with reference to former national regulations) are mentioned, regardless of the fact that some of them are not in accordance with current community law (as defined in directive 2004/24/EC). Traditional preparations were authorised in 10-50% of well-established use doses when in parallel the same preparations were authorised under well-established use.

Information on combination medicinal products

- **Austria**

A) 1 capsule containing:

- Cucurbita seed 400 mg
- Cucurbita seed oil 340 mg
- *Serenoa repens*, fruit, dry extract (7-13:1), extraction solvent ethanol 90% m/m 75 mg

Indication: Dysfunctions of the urinary tract in men

Posology: Adults: 1 capsule 3 times daily

On the market since 2008.

B) 1 capsule containing:

- Cucurbita seed 400 mg
- Cucurbita seed oil 340 mg

Indication: Dysfunction of the bladder and for facilitation of urination

Posology: Adults: 3-5 capsules daily

On the market since 2008.

- **Germany**

The main combination substances are:

Cucurbitae oleum

Sabalisa serrulatae fructus

There are 10 authorised combination products.

Number of combination substances	Number of authorised combination products
2-3	9
4-5	1
>5	0

Additional comments:

German Standard Marketing Authorisations

single active ingredient: 3 (herbal substance)

combinations products: 0

- **Hungary**

There is one combination product (with similar indications as above mentioned product containing pumpkin oil on the Hungarian market).

The main combination substances are: Cucurbitae oleum, Faex medicinalis sicc. cum zinci, Tocopherol, Faex medicinalis sicc. cum selenium.

Information on other products

- **Hungary**

There is one product of Cucurbitae pepo oleum in soft capsules 600 mg.

Indication: For decreasing symptoms associated with an enlarged prostate (irritable bladder with urgency, night urgency and urge incontinence), prevention and supportive treatment of cardiovascular disease.

Posology: Adults: 3 times 1 capsule daily

On the market since 2005.

- **Poland**

There are 3 food supplements (with similar posology, composition and pharmaceutical form as the above-mentioned product containing pumpkin oil on the Polish market).

- **Spain**

There are some products on the market sold as food. *Cucurbita pepo* seed oil in soft capsules, from 200 mg to 1,000 mg. Daily dose: 1-2 g.

Toast seeds are a common food.

1.3. Search and assessment methodology

Databases assessed up to March 2011:

Science Direct, PubMed, Embase, Medline, Academic Search Complete, Toxnet

Search terms: *Cucurbita pepo* seeds, pumpkin seeds

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Cucurbita pepo is a well-known traditional herbal medicinal product used for the centuries in Europe. Its medicinal use was described in many well-known manuscripts and textbooks. The *Cucurbita* genus comprises many species widespread in Europe, Asia and America. The genus contains about 20 species. The most popular in Central and Northern Europe are *Cucurbita pepo* L., *Cucurbita moschata* Duch. and *Cucurbita maxima* Duch. Pumpkin cultivation in Europe dates back to the ancient and medieval times.

Cucurbita has been traditionally used as diuretic and anthelmintic, and as taenifugium remedy in Europe since medieval time. Pumpkin was mentioned in the writings of Hippokrates, Dioskurides, Lonicerus (1564), Fuchs (1523), Matthiolus (1626), (according to Madaus "Lehrbuch der Biologischen Heilmittel", 1938). Images of cucurbit have been decorating the Roman villa Farnesina since 1515-1518 (Janick and Paris 2006). At that time, the first paintings of the species of New World cucurbits were known in Europe.

Traditionally pumpkin seeds were used as anthelmintic, taeniicide remedy. Their contemporary use in functional disorders of the bladder with micturition difficulties is substantiated by empirical experience.

Cucurbita pepo has been used for more than 30 years in the European Union, mainly as a remedy for various difficulties associated with an enlarged prostate gland and micturition problems related to irritable bladder (Bradley 2006; Chevallier 1996; ESCOP 2009; Martindale 2009; Gruenwald *et al.* 2000; 2004).

Not all preparations present in marketed products and reported in Table 1 fulfil the criteria for traditional use. The preparations/products found in Hungary and Poland lack evidence of a medicinal use for at least 30 years including at least 15 years in the EU.

2.2. Information on traditional/current indications and specified substances/preparations

Information on indications from products on the market

Cucurbitae semen soft extract (DER 15-25:1), extraction solvent ethanol 92% m/m for:
Relief of lower urinary tract symptoms related to benign prostatic hyperplasia (Alken stages I to II or Vahlensieck stages II to III)

Cucurbitae semen dry extract (DER 15-30:1), extraction solvent ethanol 60% v/v:
Traditionally used to strengthen bladder function

Cucurbitae oleum:

- traditionally in troubles with urination caused by benign prostatic hyperplasia
- traditionally used to strengthen bladder function

Cucurbitae semen (herbal substance and comminuted herbal substance):
Traditionally used to strengthen bladder function

Information on traditional indications from literature

British Herbal Compendium (Bradley 2006)

Pumpkin seed preparations for:

Symptomatic treatment of micturition disorders in the early stages of benign prostatic hyperplasia. Irritable bladder; functional disorders of the bladder and problems in passing water.

Commission E Monograph (published 30.11.1985, revised 17.01.1991)

Pumpkin seed (*Cucurbitae peponis semen*) for:

Relief of lower urinary tract symptoms (LUTS) related to benign prostatic hyperplasia, irritated bladder and micturition problems (Stages I to II).

This medication relieves only the symptoms associated with an enlarged prostate without reducing the enlargement. Please consult a physician at regular intervals.

ESCOP Monograph (Supplement 2009)

Pumpkin seed (or a corresponding amount of extract) for:

Symptomatic treatment of micturition disorders (dysuria, pollakisuria, nocturia, urine retention and hesitancy) in benign prostatic hyperplasia at stages I and II as defined by Alken or stages II and III as defined by Vahlensieck.

Irritable bladder.

Hager's Handbuch (Hänsel et al. 1994)

Pumpkin seeds (or equivalent preparations) for:

Lower urinary tract symptoms related to benign prostatic hyperplasia and micturition problems (Stages I to II).

Herbal Drugs and Phytopharmaceuticals (Wichtl 2004)

Pumpkin seeds for:

Supportive treatment in functional disorders of the bladder and in difficulties in passing water.

Lehrbuch der Biologischen Heilmittel (Madaus 1938)

Pumpkin seeds for:

Anthelmintic (taenifugae)

Benign prostatic hyperplasia, symptoms associated with enlarged prostate.

Martindale 2009

Pumpkin seeds formerly for:

Expulsion of tapeworms

Use as ingredient of herbal preparations used in urinary-tract disorders.

PDR for Herbal Medicines (Gruenwald et al. 2000)

Fresh and dried seeds for:

Irritable bladder and prostate complaints (this medication relieves only the symptoms associated with an enlarged prostate without reducing the enlargement).

WHO Monographs on selected medicinal plants (2009)

Pumpkin seeds (or equivalent preparations) for:

Symptomatic treatment of difficulties with micturition associated with stage I–II prostatic adenoma and irritable bladder.

In traditional medicine used for the treatment of asthma, burns, constipation, eczema, fever, tapeworms and toothache.

Assessors' comment

On the basis of the information on traditional and current indications, data on clinical efficacy (see section 4.2.) and the requirements for specified conditions of use to ensure a safe use, the following therapeutic indication is recommended for *Curcubita semen* and the preparations included in the monograph:

“relief of lower urinary tract symptoms related to benign prostatic hyperplasia or related to an overactive bladder, after serious conditions have been excluded by a medical doctor”.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

Information on posology, route of administration, duration of use from products on the market

Cucurbitae semen soft extract (DER 15-25: 1), extraction solvent ethanol 92% m/m:
500 mg twice daily

Cucurbitae semen dry extract (DER 15-30: 1), extraction solvent ethanol 60% v/v:
105 mg 3 times daily (hard capsule)
152 mg twice daily (film-coated tablet)

Cucurbitae oleum:
1166 mg 3 times daily
1000 mg, 3-4 times daily

Cucurbitae semen (herbal substance):
Daily dose up to 20 g

Long-term use is possible (longer than 4 weeks).

Information on posology, method of administration, duration of use from literature for relevant preparations

British Herbal Compendium (Bradley 2006)

Daily dose: 10–30 g of pumpkin seed, as ground seed or an equivalent amount of ethanolic extract or pumpkin seed oil.

Duration of use: Pumpkin seed preparations should be taken for at least several weeks or months to achieve optimum effects.

Commission E Monograph (published 30.11.1985, revised 17.01.1991)

An average daily dose: 10 g of seeds or equivalent preparations of the whole and coarsely ground seed and other galenical preparations for internal use.

Duration of use: not specified

ESCOP Monograph (Supplement 2009)

Daily dose: 10-20 g of the seeds or a corresponding amount of an extract.

Duration of use: No restriction. Long-term administration may be advisable. If symptoms persist or worsen, medical advice should be sought.

Hager's Handbuch (Hänsel et al. 1994)

Dosage: pumpkin seeds, 1 – 2 heaped table spoonful (15-30 g) taken with fluid, morning and night. An average daily dose: 10 g of seeds or equivalent preparations. 1 teaspoon of seeds 3 times daily.

Duration of use: No restriction. Long-term administration may be advisable. If symptoms persist or worsen, medical advice should be sought.

Herbal Drugs and Phytopharmaceuticals (Wichtl 2004)

Dosage: Ground or chewed pumpkin seeds, 1 – 2 heaped table spoonful (15-30 g) taken with fluid, morning and night.

Duration of use: For the drug to work, experience indicates that it has to be taken for a period of weeks or months.

Lehrbuch der Biologischen Heilmittel (Madaus 1938)

Dosage: 1 teaspoon of seeds 3 times daily.

Duration of use: no information

PDR for Herbal Medicines (Gruenwald et al. 2000)

Daily dosage: The average daily dosage is 10 g of ground seeds; 1 to 2 heaping dessert spoons with liquid in the morning and evenings.

Duration of use: no information

WHO Monographs on selected medicinal plants (2009)

Dosage forms: Crude drug and extracts. Oral daily dose: 10 g of seed; equivalent preparations.

Duration of use: no information

Assessors' comment

On the basis of the information on traditional and current dosages, information on duration of use from clinical studies (see section 4.2.2.) and the requirements for specified strength and specified posology, the following is recommended for Cucurbitae semen and the preparations included in the monograph:

Posology

Adults and elderly

Herbal substance

Single dose: 2.5 – 7.5 g, 2 times daily. Daily dose up to 20 g

Herbal preparations

a) Comminuted herbal substance

Single dose: 2.5 – 7.5 g, 2 times daily.

b) Soft extract

Single dose: 500 mg, 2 times daily.

c) Dry extract

Single dose: 105 mg, 3 times daily
or 152 mg, 2 times daily

d) Fatty oil

Single dose: 1 - 1.2 g, 3 times daily
Daily dose: 3 – 4 g

The use in children and adolescents under 18 years of age is not recommended because lower urinary tract symptoms in these populations require medical supervision.

Duration of use

Long-term use is possible.

3. Non-Clinical Data

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

***In vitro* experiments**

Antioxidant activity

Four types of commercially available pumpkin seeds (*Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita mixta*) from Bulgaria, China and Turkey have been subjected to two types of extraction to get the total lipid fraction or separate the individual ingredients according to their polarity. In the Extraction No. 1 (1:5) procedures were made using solvents with successively decreasing the polarity: acidified water, methanol, acetone, and ethylacetone. In the Extraction No. 2 (1:5), total lipids were extracted by Folch method using chloroform : methanol (2:1, v/v). Then, total lipids were separated using counter-current distribution. The final extracts were screened for their antioxidant activity and their inhibitory activity against lipid peroxidation. The results show that radical scavenging activity of the water extracts was related to their total phenolic content (up to 85-92% of total extractable phenolics). The highest antioxidant activity (with EC₅₀ values in the range of 4.51 to 6.71 mg/ml) was found for water and methanol fractions, the lowest for ethyl-acetate fractions. Pumpkin seed water extracts inhibited lipid peroxidation at 1.5 mg/ml, while the acetone extracts inhibit 50% of lipoxygenase activity at the range from 0.16 to 0.80 mg/ml. The phenolic fraction did not determine activity of the extracts against lipoxygenase. This discrepancy is probably due to the fact, that radical scavenging is just one way by which molecules can inhibit lipoxygenase (Xanthopoulou *et al.* 2009).

Antiandrogenic activity

An ethanolic extract (DER 2:1) of *Cucurbita pepo* seeds containing mainly triglycerides, Δ^5 – and Δ^7 -sterols and tocopherol did not show antiandrogenic activity. Experiments were performed *in vitro* by use of an androgen receptor responsive reporter gene assay (Schleich *et al.* 2006).

Schmidlin and Kreuter (2003) described the influence of a *Cucurbitae pepo* seed extract on the activity of aromatase and 5- α -reductase Typ II in the homogenates of human and rat placenta. The pumpkin extract was as well tested in an experimental model of the prostate hypertrophy induced by injection of testosterone in Sprague-Dawley castrated male rats. The incubation of human placenta homogenate with 10 mg/ml of the extract induced 44.7 \pm 0.2 – 55.9 \pm 15.7% reduction of the activity of aromatase. The activity of the enzyme 5- α -reductase Typ II in human placenta homogenate was inhibited at 90.4 \pm 2.4%, 71.4 \pm 4.1% and 29.6 \pm 7.9% respectively by 10 mg/ml, 2 mg/ml and 0.4 mg/ml concentrations of the extract. The activity of this enzyme in rat placenta homogenate was inhibited at 69.7 \pm 2.8%, 27.0 \pm 1.5.1% and 6.5 \pm 1.1% respectively by 10 mg/ml, 2 mg/ml and 0.4 mg/ml concentrations of the extract. The activity of the same enzyme in human embryonic kidney (HEK 293) cells was inhibited at 52.0 \pm 2.4%, 28.0 \pm 2.4.1% and 14.8 \pm 7.8% respectively by 50 mg/ml, 20 mg/ml and 5 mg/ml concentrations of the extract. Testosterone (1 mg/kg) injection in castrated rats after 4 days induced an increase of the weight of the prostate gland to 41 \pm 1.9 mg/100 g kg from 13.7 \pm 1.9 mg/100 g kg (100% arbitrary reduction). After subcutaneous finasterid injection (1 mg/kg) in the

positive control group the reduction of the weight of prostate glands was 76% ($p < 0.01$, $n = 7$) while in the pumpkin extract group (100 mg/kg, for 4 days, $n = 7$) the decrease of weight was 31%.

Inhibition of enzymatic activity

Ribosome-inactivating proteins (RIPs) are considered to be plant defence-related proteins against several pathogenic viruses, fungi, and bacteria. Regardless of a significant level of RIP in the materials analysed (*Cucurbitaceae*, *Allium cepa*, *Daucus carota*, spinach), which are eaten raw by humans and animals, those levels of RIP are not harmful. Presence of moschatin and peponin in edible parts of vegetables (also in *Cucurbita pepo* seeds), due to their antibacterial and antiviral activity could even be beneficial (Barbieri *et al.* 2006; Gerhäuser *et al.* 1993).

Ribonuclease from pumpkin seeds (*Cucurbita* sp.) showed a potent RNase activity against baker's yeast RNA and calf liver RNA. Furthermore, it was able to hydrolyse total RNA of both animal and plant source (Fang *et al.* 2010).

Many microbial and insects proteases which facilitate invasion of plants have trypsin like features. Plants have a defense strategy against proteolytic digestion of proteins (Habib and Fazili 2007). Inhibitors of trypsin, activated Hageman factor (a serine protease implicated in blood coagulation), human leucocyte elastase and cathepsin G inhibitors from *Cucurbita pepo* seeds and other members of the *Cucurbitaceae* present antitrypsin activity. The degree of enzymatic activity in the seeds of some *Cucurbitaceae* plants varied between 31 and 399 units/100 g of fresh tissue (Bombardelli and Morazzoni 1997). Trypsin inhibitors activity in *Cucurbita pepo* and *Cucurbita moschata* seeds was low as compared to Glycine max activity (Henderson *et al.* 1986).

Wang and Ng (2003) revealed the presence of the cucurmoschin, a peptide with antifungal properties from the seeds of *Cucurbita moschata*.

Immunological activity

Three different extracts of pumpkin seeds suppressed dose-dependently mitogen-induced neopterin production and tryptophan degradation induced by cytokine interferon- γ (IFN- γ) (Winkler *et al.* 2005). Pumpkin seeds of *Cucurbita pepo citrullina* I. Greb. var. *styriaca* I. Greb. from biological cultivation were finely grounded (10 g of the powder was added to 100 ml of Roswell Park Memorial Institute medium (RPMI 1640)). The cold extract was shaken for 10 minutes; the hot extract was boiled for 1 minute. A third extract was prepared from pumpkin seeds (1 capsule – 0.72 g). Unfortunately, there is a lack of precise information about the substance contained in the capsule. Peripheral blood mononuclear cells (PMBC) were isolated from the whole blood from healthy human donors. The PMBC were stimulated with phytohaemagglutinin and concanavalin A as controls. In mitogen-stimulated cells neopterin concentrations declined significantly dose-dependently after 48 hours co-incubation of 1 ($p < 0.05$), 10 ($p < 0.01$) and 50 ($p < 0.001$) mg/ml concentrations of the cold and hot extracts and 12 $\mu\text{g/ml}$ ($p < 0.001$) of the capsules compared to control.

All extracts were correspondingly effective to suppress tryptophan degradation and stimulation induced neopterin formation. Kynurenine to tryptophan ratio, dose-dependently significantly declined in PMBC coincubated for 48 hours with three extracts, stimulated with phytohaemagglutinin and concanavalin A as controls. The hot and cold extracts similarly influenced the formation and release of the cytokine IFN- γ in mitogen stimulated PMBC's, whereas the extract from capsules appeared to be less effective.

Antiviral activity

Peponin, ribosome inactivating protein (RIP Type I) from the seeds of *Cucurbita pepo*, showed dose dependently inhibitory activity of cell protein synthesis and human immunodeficiency virus Type 1 reverse transcriptase (HIV-1 RT). The ribosome inactivation was established using cell-free *in vitro* translation system with use of nuclease treated rabbit reticulocyte lysate and Tobacco mosaic virus

(TMV) RNA. Peponin, which induced inhibition of cell-free protein synthesis ($ID_{50}=5.4\pm 1.4$ ng/ml, $n=4$), was found to be a strong inhibitor of HIV-1 RT ($ID_{50}=12.7\pm 0.31$ μ g/ml, $n=2$) (Gerhäuser *et al.* 1993).

Antifungal activity

Cucurmoschin, a peptide with antifungal properties from the seeds of *Cucurbita moschata* was active against *Botrytis cinerea*, *Fusarium oxysporum*, *Mycosphaerella arachidicola* (Wang and Ng 2003; Ng 2004). A slight inhibition of mycelial growth was found on Petri plates at 75 μ g, and was evident at a dose of 375 μ g. Cucurmoschin was also assayed for translation-inhibiting activity in rabbit reticulocyte lysate system. The peptide inhibited cell-free translation with an IC_{50} of 1.2 μ M.

Different basic polypeptides from the soluble and cell wall-derived fractions of *Cucurbita maxima* seeds (Vassiliou *et al.* 1998) were isolated using HPLC methods. Fractions containing the major P2249, P4650 and P11696 Da components exhibited antifungal activity. The antifungal activity of proteins from *Cucurbita maxima* seeds was determined using growth curves after 60 hours of incubation using fixed protein concentration (Table 4).

Table 4. Antifungal activity of the *C. maxima* seed P2249, P4650 and napin-like complex P11696 proteins (Vassiliou *et al.* 1998)

Fungus	IC ₅₀ (μ g/ml) or (% inhibition, AFP concentration (μ g/ml))		
	Peak 0 (P2249)	Peak 2 (P4650)	Peak 4 (P11696)
<i>A. brassicicola</i>	64	34	19
<i>C. elegans</i>	15	5	7
<i>F. oxysporum</i>	(63%, 40)	(59%, 61)	(51%, 59)
<i>B. cinerea</i>	(0%, 27)	(0%, 61)	(0%, 70)
<i>V. dahliae</i>	(23%, 68)	(26%, 42)	(45%, 59)
<i>F. proliferatum</i>	(0%, 68)	(0%, 42)	(0%, 59)
<i>S. cerevisiae</i>	(30%, 45)	(21%, 47)	(50%, 47)

(IC_{50} values) = protein concentrations required for 50% inhibition of growth after about 60 hours of incubation

AFP = antifungal proteins

Protein concentrations required for 50% inhibition of growth after about 60 hours of incubation (IC_{50} values) were determined from the dose-response curves (percentage of growth inhibition plotted against protein concentration). Where dose-response curves have not been determined, percentage growth inhibition after 60 hours is given in brackets, together with the concentration of antifungal protein preparation used, in μ g/ml. The major components of the particular peak fractions used are indicated in parentheses. Peak 0 (major component P2249), peak 2 (major component P4650) and peak 4 (major component P11696) were used. The P4650 preparation (peak 2) contained minor amounts of P4779 and other homologues of P4650.

Antiparasitic activity

The antifungal protein from pumpkin *Cucurbita moschata* tested *in vitro* against the plant parasitic nematodes *Ditylenchus dipsaci* and *Heterodera glycines* had very low anti-nematode potencies and could be considered as practically inactive (Zhao *et al.* 2009).

Marie-Magdeleine *et al.* (2009) tested *in vitro* activity of three extracts of *Cucurbita moschata* seeds on four developmental stages of a parasite of small ruminants, the nematode *Haemonchus contortus*. From the three extracts (water, methanol and dichloromethane), only the aqueous extract inhibited adults worm motility (> 59.2% after 24 hours of incubation). All tested extracts strongly inhibited larval development (> 90% for each extract, $p < 0.001$).

***In vivo* experiments**

Influence on urinary functions

The effects of an unspecified water soluble extract of pumpkin seeds and soybean germ extract on in-bladder pressure (cystometrogram) and urination frequency of male rats were tested (Hata *et al.* 2005). Pumpkin seed water-soluble extract (250 mg/kg) compared to control solvent (1% dimethyl sulfoxide diluted in sterile physiological saline) and soybean germ extract significantly increased bladder volume, decreased urination frequency and increased urination delay index (Table 5. and Table 6.) (Hata *et al.* 2005). According to the authors, the observed effects of the relaxation of the bladder and decrease of in-bladder pressure are related to the increased productions of NO via the arginine/NO pathway.

Arginine is present in the pumpkin seed extract in two-fold the concentrations of other amino acids. It was suggested that arginine/NO metabolism, independently of adrenaline and acetylcholine, is involved in relaxation of urination muscle at a stage of full bladder (Andersson and Wein 2004).

Table 5. Urination frequency (times/min) (Hata *et al.* 2005)

Sample name	Dose N	Before administration	After solvent administration	After test sample administration
Pumpkin seed water-soluble extract (Lot No. 3038141)	250 mg/kg 3	1.62±0.38	1.51±0.20	0.58±0.14 ^{*#}
Pumpkin seed water-soluble extract (Lot No. 3036525)	250 mg/kg 4	2.61±0.66	2.41±0.43	1.13±0.27 ^{&@}
Soybean germ extract	250 mg/kg 3	1.61±0.33	1.51±0.30	1.51±0.13

* With statistical significance compared to before administration ($p < 0.05$)

With statistical significance compared to before administration ($p < 0.001$)

& With statistical significance compared to after solvent administration ($p < 0.005$)

@ With statistical significance compared to after solvent administration ($p < 0.001$)

Table 6. Urination delay index with the urination frequency before administration as one (-fold) (Hata *et al.* 2005)

Sample name	Dose N	Before administration	After solvent administration	After test sample administration
Pumpkin seed water-soluble extract (Lot No. 3038141)	250 mg/kg 3	1.00	1.06±0.15	2.96±0.1.19 [*]

Sample name	Dose N	Before administration	After solvent administration	After test sample administration
Pumpkin seed water-soluble extract (Lot No. 3036525)	250 mg/kg 4	1.00	1.08±0.19	2.33±0.35 ^{#@}
Soybean germ extract	250 mg/kg 3	1.00	1.51±0.30	1.51±0.13

* With statistical significance compared to before administration ($p < 0.05$)

With statistical significance compared to before administration ($p < 0.001$)

@ With statistical significance compared to after solvent administration ($p < 0.001$)

Influence on prostate gland

Pumpkin seeds alleviated the signs of experimentally induced BPH in rats such as decrease of protein binding prostate (PBP) levels, size of the prostate and improved the histology of testis. Pumpkin seeds given orally (2.5, 5 and 10% in a diet) dose-dependently inhibited citral-induced hyperplasia of the prostate, especially at high concentration seed dose (10%, $p < 0.02$) (Abdel-Rahman 2006).

In experimental model of hyperplasia of the prostate in rats, Gossell-Williams *et al.* (2006) tested the therapeutic effects of pumpkin seed oil. Hyperplasia of prostate gland was induced by a subcutaneous injection of testosterone: 0.3 mg/100 g of body weight (b.w.)/day for 20 days. The 1st group of tested rats received simultaneous administration of testosterone and pumpkin oil. The 2nd group received simultaneous administration of testosterone and corn oil for 20 days, the 3rd group only corn oil for 20 days. Pumpkin seed oil was administered in doses of 2.0 or 4.0 mg/100 g of body weight for 20 days. After autopsy on day 21, the prostate of each rat was weighed, and the prostate size ratio (weight of prostate/b.w. of the rat) was established. Testosterone significantly increased prostate size ratio ($p < 0.05$) and this increase was significantly inhibited by treatment with pumpkin seed oil at 4.0 mg/100 g b.w.

Tsai *et al.* (2006) tested pumpkin seed oil efficacy for 14 days, in experiments performed in rats on the model of prostatic growth induced by subcutaneous daily injection of testosterone (1.25 mg/kg/day) together with prazosin (30 µg/kg/day) (T-P). Pumpkin seed oil (PSO) (2.5 ml/kg/day) extracted from pumpkin seeds was administered concomitantly together with T-P. As compared with T-P alone group, the T-P group treated with PSO had significant lower weight ratio for ventral prostate ($p = 0.01$) and lower protein levels within ventral lobe and dorsolateral lobe ($p = 0.03$ and $p = 0.003$, respectively).

Assessors' comment

In experimental animal models of prostatic growth pumpkin seeds and pumpkin seeds oil significantly reduced weight of prostate and lowered protein levels, however the doses used were comparatively higher than the equivalent used in humans for therapeutic purpose.

Urodynamic effects

The urethral and bladder pressure were determined in anesthetized rabbits before and after administration of the extracts of pumpkin oil, n-butanol and ether fractions. The dose injected daily for 7 days was equivalent to 45 g of pumpkin seed. The bladder pressure was measured before and at 30, 60, 120 and 180 minutes after injection. Statistically significant ($p < 0.05$) decrease of bladder and urethral pressure was registered only after oil fraction injections. The decrease of bladder pressure was 7.6 mm Hg ($p < 0.001$) with maximum effect after 2 hours, and urethral pressure 5.4 mg Hg ($p < 0.01$)

as compared to pre-treatment values. The administration of the n-butanol and ether fraction had no significant influence (Zhang *et al.* 1994).

Cardiovascular activity

The dried powdered seeds of *Cucurbita maxima* were extracted with 80% of ethanol, the filtrate was evaporated and the residue (PE) was dissolved in distilled water (concentration 500 mg/ml). The extract induced a positive inotropic effect on frog's heart. Small short lasting rise in mean arterial pressure, elevation of QRS complex and reduction of heart rate in a dog was also observed (Lahon *et al.* 1978).

In spontaneously hypertensive rats treated with pumpkin seed oil for 4 weeks (40 mg/kg), significant retardation of progress of hypertension with concomitant administration of felodipine or captopril was observed (Al-Zuhair *et al.* 2000).

Anti-inflammatory activity

Fahim *et al.* (1995) described an anti-inflammatory activity of pumpkin seed oil administered in intramuscular injection in experimental arthritis in male Sprague-Dawley rats. Experimental arthritis was induced by inoculation of Freund's complete adjuvant to the subplantar surface of the hindpaw. Pumpkin seed oil (100 mg/kg b.w.) was administered for 7 days before the adjuvant injection and then up to 22nd day afterwards. For comparison, other groups of rats received in the same order: indomethacin (2 mg/kg b.w.), pumpkin seed oil + indomethacin and control group: 1% Tween 80. Both pumpkin seed oil and indomethacin were suspended in 1% Tween 80. During the acute and chronic phase of inflammation blood samples were collected for determination of blood glutathione (GSH), plasma total proteins, albumin serum sulfhydryl group (SH-gps), ceruloplasmin (CP) and lysosomal marker – N-acetyl- β -D-glucosaminidase (NAG). After completing the experiment, liver samples were used for determination of glucose-6-phosphate dehydrogenase (G-6-P DH) activity and protein content of liver homogenates was established. Adjuvant inoculation resulted in decrease of serum SH-gps, with an increase of serum CP reduction of blood glutathione and total proteins and albumins levels. Liver G-6-P DH activity was markedly increased. The treatment with pumpkin seed oil resulted in normalization of altered parameters, notably in chronic phase, except serum NAG influence. Pumpkin oil administration inhibited paw oedema during the chronic phase in about 44% as compared to the control untreated group. It reduced also liver G-6-P DH activity to almost 50% of the arthritic groups' level. No potentiation of the antiinflammatory effects of indomethacin combined with pumpkin seeds oil was observed.

Antioxidant activity

Pumpkin seed protein isolate antioxidant activity was tested in the model of CCl₄ intoxication in low-protein fed Sprague-Dawley rats (80-90 g b.w.) (Nkosi *et al.* 2005; 2006a). The dried dehusked and defatted powder of *Cucurbita pepo* seeds was suspended in distilled water (pH 10) and filtered. The filtrate was centrifuged; pH adjusted to 5 and residue was freeze-dried. Experimental groups of Sprague-Dawley rats were kept on low-protein diet for 5 days and underwent experimental hepatic injury with i.v. injection of CCl₄. Two hours after the CCl₄ administration, one group of rats received 1 ml/kg b.w. of the pumpkin seed protein isolate in saline (20 g/100 ml) by gavage. Autopsy was performed at 2, 24, 48 and 72 hours after the CCl₄ intoxication and plasma samples and liver tissue were tested. CCl₄ intoxication resulted in increase in lipid peroxidation (LPO) and decreased activity of glucose-6-phosphatase (G-6-Pase), catalase (CA), superoxide dismutase (SOD), glutathione peroxidase (GSHpx), and significant decrease of the total antioxidant capacity (TAC). Lipid peroxidation levels induced by CCl₄ intoxication resulted in significantly increased lipid peroxidation (LPO) levels in liver homogenates (p<0.05). Pumpkin seed protein isolate administration significantly increased the level of CA enzyme at 48 and 72 hours after treatment (p<0.05). Plasma SOD activity was significantly

increased, at all intervals tested ($p < 0.05$). Moreover, GSHpx activity was significantly increased ($p < 0.05$) and already higher than that of untreated control rats besides intoxicated CCl_4 animals. Overall the TAC was significantly increased at all intervals tested ($p < 0.05$). G-6-Pase levels in the liver homogenates were elevated over all intervals tested and neutralization of the effects of CCl_4 was registered for G-6-Pase 72 hours after treatment ($p < 0.05$). LPO activity of the liver microsomes was significantly reduced after pumpkin seed protein isolate administration ($p < 0.05$).

Similar experiments were performed by Nkosi *et al.* (2006b) illustrating antioxidative effects of the pumpkin seed protein isolate against acetaminophen induced liver injury in low protein fed rats (Sprague Dawley, 80-90 g b.w). The dried dehusked and defatted powder of *Cucurbita pepo* seeds was suspended in distilled water (pH 10) and filtered. The filtrate was centrifuged; pH adjusted to 5 and residue was freeze-dried. Experimental groups of Sprague-Dawley rats were kept on low-protein diet for 5 days and underwent experimental hepatic injury with subcutaneous injection of 600 mg of acetaminophen /10 ml PEG 400: physiological saline (1:1)/kg b.w. The control group received 10 ml/kg polyethylene glycol 400 (PEG 400: physiological saline (1:1)/kg b.w. Two hours after the acetaminophen administration, one group of rats received 1ml/kg b.w. of the pumpkin seed protein isolate in saline (20 g/100 ml) by gavage. Autopsy was performed at 2, 24, 48 and 72 hours after the acetaminophen intoxication and plasma samples and liver tissue were tested. In initial experiment *in vitro* the total polyphenol (2.3 mg/g) and sulfhydryl (1.39 $\mu\text{g}/\text{mg}$) content was measured as indicative of potential antioxidative properties. At a concentration of 0.1 g/ml, the pumpkin seed protein isolate exhibited about 80% free radical scavenging ability. The pumpkin seed protein isolate administration induced a strong chelating activity of approximately 64% on Fe^{2+} ions at a concentration of 0.5 g/ml. However the protein isolate was found weak in preventing the generation of free radicals (only 10% inhibition of xanthine oxidase). The pumpkin seed protein isolate administration resulted in significant decrease of activity of the aspartate transaminase (AST) and alanine transaminase (ALT) in acetaminophen elevated plasma activity levels of both enzymes ($p < 0.05$). The presence of sulfhydryl groups could be related to replenishing the depleted thiol groups by acetaminophen. The pumpkin seed protein isolate was found to induce better protection against acetaminophen toxicity than carbon tetrachloride toxicity.

Hypolipidemic effects

In dietary induced hypercholesterolemia in rabbits pumpkin seed oil (40 mg/kg b.w.) given together with simvastatin for three weeks significantly attenuated increased aortic contractile response to norepinephrine and prevented elevated activity of serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and creatine phosphokinase (CPK). The administration of simvastatin as solitary treatment induced a significant increase of activity of ALAT, ASAT and CPK (Al Zuhair *et al.* 1997). This experiment exhibited the potentiation of the antihypercholesterolemic effect of statins with a possible future application to decrease their therapeutic dose together with a reduction of the number of side effects.

Four groups of twenty recently weaned male Wistar rats received for 10 days commercial whole, sifted (sieve 0.5 mm) and residual pumpkin seed flour to maintain the rate of 30% of the total starch and dextrin in the control group (Cerqueira *et al.* 2008). Blood samples have been evaluated in 48 hours intervals to measure cholesterol, triacylglycerides and glucose levels. The ingestion and animals growth were similar in all groups along experiment ($p > 0.05$). Glucose levels were significantly decreased in group treated with whole pumpkin seeds, the level of triacylglycerides was significantly lowered in the group receiving sifted pumpkin flour ($p < 0.05$) (Table 7).

Table 7. Levels of glucose, triacylglycerides and cholesterol after oral administration for 10 days of pumpkin seed flour to recently weaned male Wistar rats (Cerqueira *et al.* 2008).

Biochemical results	Experimental groups (mg/dL)			
	Control	Pumpkin seed flour		
		Whole	Sifted	Residual
Glucose	82.50	32.43*	58.31	52.43
Triacylglycerides	150.80	113.33	82.13*	89.33
Cholesterol total	105.55	95.30	78.25	67.45

* p<0.05 compared to control group

Makni *et al.* (2008) administered a flax and pumpkin seed mixture for 30 days in experimental model of dietary hypercholesterolemia in rats. The seed mixture was rich in ω -3 and ω -6 unsaturated fatty acids with ratio of ω -3/ ω -6 as 5/1. One group of rats received control (CD) nutrient diet for rodents, the second group received a diet supplemented with 1% of cholesterol (CD-chol) and the third group group CD diet supplemented with seed mixture powder substituted at 33% and with 1% of cholesterol (MS-chol). One month's treatment resulted in a significant decrease of lipid parameters in the MS-chol group compared to the CD-chol group (Table 8). Moreover, plasma and liver fatty acid composition showed an increase of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and a decrease of saturated fatty acids (SFA). Histological sections of the liver showed lipid vacuolization in hepatocytes of CD-chol group and improvement in MS-chol group.

Table 8. Plasma and liver lipid profile in CD, CD-chol and MS-chol groups (Makni *et al.* 2008)

Parameters and treatments	CD	CD-chol	MS-chol
<i>Total lipid</i>			
Plasma(mg/ml)	9.77±0.38	16.18±0.62***	14.38±0.56 ⁺
Liver(mg/g)	95.35±1.06	134.9±0.42***	113.83±3.15 ⁺⁺
<i>Total cholesterol</i>			
Plasma (g/l)	0.69±0.05	1.08±0.17***	0.80±0.11 ⁺⁺
Liver (mg/g)	10.09±0.45	12.14±0.22 ^{**}	10.670.38 ⁺⁺
<i>Triacylglycerol</i>			
Plasma (g/l)	0.66±0.09	1.05±0.07***	0.64±0.06 ⁺⁺
Liver (mg/g)	11.12±0.15	12.49±0.33 [*]	10.19±0.44 ⁺⁺
LDL-cholesterol(g/l)	0.25±0.07	0.63±0.18***	0.37±0.12 ⁺⁺
HDL-cholesterol(g/l)	0.30±0.02	0.23±0.02 ^{**}	0.30±0.05 ⁺⁺
HTR (%)	43.47±0.04	21.29±0.09***	37.50±0.12 ⁺⁺⁺
Atherogenic index (AI)	1.27±0.04	3.62±0.10***	1.66±0.08 ⁺⁺⁺
LDL/HDL ratio	0.83±0.09	2.72±0.14***	1.22±0.12 ⁺⁺⁺

HTR(%)=HDL-C/TC ratio

Values are given as means ± standard deviation (mean of six determinations)

Significant differences between the CD-chol and CD groups: *p<0.05; **p<0.01; p***p<0.001

Significant differences between the MS-chol and CD-chol groups: ⁺p<0.05; ⁺⁺p<0.01; ⁺⁺⁺p<0.001

In an oestrogen deficiency model in female ovariectomized Sprague-Williams rats, pumpkin seed oil (PSO) from *Cucurbita maxima* and *Cucurbita stilbo* was administered for 12 weeks to test influence on the plasma lipid profile and cardiovascular parameters (Gossell-Williams *et al.* 2008). After cold pressing the stock oil, which contained 930 mg/ml, was diluted to a concentration of 80 mg/ml with corn oil as vehicle. One week later ovariectomy rats diet were supplemented either with 40 mg/kg body weight of PSO or with corn oil (CO, vehicle for PSO). Rats were divided into four groups (N=5–6): non-ovariectomized rats fed PSO (control PSO) or CO (control CO) and ovariectomized rats fed PSO (OVX/PSO) or CO (OVX/CO). Animals received supplementation 5 days weekly for 12 weeks (Table 9).

Table 9. Plasma lipid concentration after 12 weeks of treatment (Gossell-Williams *et al.* 2008)

Plasma lipids (mg/dl)	Control CO (n=6)	Control PSO (n=5)	OVX/C(n=6)	OVX/PSO (n=5)
TC	56.10±2.90	38.64±2.71 ^a	68.57±2.59	44.28±3.36 ^b
HDL-C	16.63±1.99	25.34±1.64 ^a	6.28±1.65 ^a	21.88±1.36 ^b
Triglycerides	76.73±4.21	58.42±2.99 ^a	94.58±8.32 ^a	61.46±2.01 ^b
LDL-C	21.90±3.48	7.78±2.26 ^a	39.4±2.81 ^a	10.59±2.36 ^b

^ap<0.001 when compared with the control CO group and ^bp<0.001 when compared with the OVX/CO group.

TC – Total cholesterol

Both control rats and ovariectomized receiving pumpkin oil were characterized by significantly lower values of total cholesterol, triglycerides and LDL, and elevated levels of HDL. The OVX/PSO group had lower diastolic blood pressure over 12 weeks of treatment than the OVX/CO group (p<0.001).

Hepatoprotective activity

Protective effects of pumpkin seed protein isolate against liver damage induced by CCl₄ in low-protein fed rats was estimated by Nkosi *et al.* (2005). The dried dehulled and defatted powder of *Cucurbita pepo* seeds was suspended in distilled water (pH 10) and filtered. The filtrate was centrifuged; the pH adjusted to 5 and the residue was freeze-dried. Experimental groups of Sprague-Dawley rats were kept on low-protein diet for 5 days and underwent experimental hepatic injury with IV injection of CCl₄. Two hours after the CCl₄ administration, one group of rats received 1 ml/kg b.w. of the pumpkin seed protein isolate in saline (20 g/100 ml) by gavage. Autopsy was performed at 2, 24, 48 and 72 hours after the CCl₄ intoxication and plasma samples were tested. The significant increase of activity levels of lactate dehydrogenase (LD), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in plasma samples was registered in control intoxicated rats. Pumpkin seed protein isolate administration significantly reduced the level of LD, ALT, AST and ALP at all intervals tested (p<0.005). Neutralization of the effects of CCl₄ was registered for LD 48 hours after treatment, and for AST, ALT and ALP 72 hours after intoxication (p<0.05).

Antiparasitic activity

Cucurbita maxima seed powder administered for 10 days (50, 100 and 200 mg/kg of feed) in domestic fowl did not result in any significant anthelmintic activity. In another experiment only in 8.69% a removal of *Raillietina cesticillus* cestodes after feeding the birds with 2 ml of an ethanolic (80%) extract (300 mg/ml) was observed. The dried powdered seeds of *Cucurbita maxima* were extracted with 80% of ethanol, the filtrate was evaporated and the residue (PE) was dissolved in distilled water (concentration 500 mg/ml) (Lahon *et al.* 1978).

The amino acid cucurbitine, isolated from *Cucurbita moschata* Duch. was administered orally to mice for 28 days since they were infected with 58-62 cercariae of *Schistosoma japonicum* (Shu-hwa *et al.* 1962). Cucurbitine at the doses of 100, 200, 300, 400 and 500 mg/kg/day for 4 weeks, starting from the day of exposure infection, resulted in decreased average number of worms (26.4, 25.3, 18.0, 11.8, 8.8 respectively) while that of the control infected group was 34.0 ($p < 0.05$). When cucurbitine was administered 2-3 weeks after exposure, the prophylactic effect of the treatment was not shown. Cucurbitine administration in the dose of 300-400 mg resulted with a worm reduction rate of about 50% and retardation of their development.

Oral administration of powdered pumpkin seeds was tested in experimental infection of nodular worm (*Oesophagostomum* spp.) in piglets (Mägi *et al.* 2005). Groups of 4 crossbred nematode free piglets of both sexes with an average weight of 13.3 kg were inoculated per os by syringe with 5,000 larvae of *Oesophagostomum* spp. Each group received 5 g per kg of body weight of some medicinal plants, pumpkin seeds included, three times at weekly intervals after start of patency. For comparison, one piglet group was treated with 1% ivermectin. The test was terminated with autopsy and the worms were recovered from intestine by the agar-gel migration technique, counted and identified. The pumpkin seeds treatment demonstrated the nematicidal effect as the number of worms recovered and excreted eggs was significantly lower compared to control untreated pigs. Moreover, therapeutic effects of pumpkin seeds were better than ivermectin administration (Table 10).

Table 10. Nematicidal effect of powdered pumpkin seeds in piglet compared to ivermectin and a non-treated control (Mägi *et al.* 2005)

Treatment and dose	EPG (after treatments)	Worm burden at autopsy	Reduction %
Pumpkin seeds 5 g/kg	80	105	97.5
Ivermectin 1% 1 ml/33kg	200	166	96.1
Non-treated control	3720	4270 (100%)	0

EPG (the number of excreted eggs in one gram of faeces)

Mahmoud *et al.* (2002) described therapy after experimental infection with the trematode *Heterophyes heterophyes* in dog puppies with decoctions of pumpkin seeds and areca nuts. Decoctions of pumpkin seeds were prepared by boiling 10 g of grounded seeds in 15 ml of water for about an hour, decoctions of areca nuts by boiling 5 g in 15 ml of water. Both decoctions were orally administered, daily for 2 weeks after start of the infection (extract of pumpkin seeds: 10 g, extract of areca nuts: 5 g). In the group receiving an extract of pumpkin seeds, the deformation of eggs started on the 4th day of treatment, however the complete destruction of eggs and eradication of adult worms were acquired with combined extract therapy.

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

No data are available on pumpkin seeds pharmacokinetics due to its complex phytochemical composition.

Data on cucurbitine

Pharmacokinetics was tested in male mice after oral or intraperitoneal administration of [¹⁴C] labelled cucurbitine (100 – 200 mg/kg). The highest levels of compound were found in liver and kidney but after 24 hours concentrations of the drug in tissues did not significantly differ. The drug was excreted mainly by kidney and marginally with faeces. Urine analysis showed that 97% of radioactivity was cucurbitine. Autoradiography performed in mice with [¹⁴C] labelled cucurbitine showed that radioactivity was highly localized in the liver, kidney, dorsal root ganglion, tracheal cartilage and pancreas at 20 minutes after i.v. injection (Liang *et al.* 1982).

Due to lack of human data on pharmacokinetics, no general conclusions can be drawn.

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

Genotoxicity

No scientifically reliable data could be found on the genotoxicity of pumpkin seeds and the pumpkin seeds preparations.

Carcinogenicity

No published data could be found on the carcinogenicity of the pumpkin seeds and the pumpkin seeds preparations.

Cytotoxic activity

The inhibition of protein synthesis in the rabbit reticulocyte lysate by pumpkin seed extract was measured (Gasperi-Campani *et al.* 1977). The concentration of the extract which inhibited protein synthesis by 50% (ID₅₀) was 67.4 µg/ml. The extract was prepared by shelling the seeds and grounded 5–8 times with 4–5 vol. ethyl ether. The resulting powder was dried, mixed with 10 volumes of cold 0.2 M NaCl, containing 0.005 M sodium phosphate buffer for 3 hours, then centrifuged at 20 000 times for 20 minutes and the supernatant was referred as a crude extract. The extract from *Cucurbita pepo* was found to have RNAase activity and was partially heat resistant.

Ribosome-inactivating proteins (RIPs) possess capability to inactivate ribosomes by modifying the rRNA. Such mechanism is responsible for their cytotoxic effect, as a result of necrosis or apoptosis. Cytotoxic activity of moschatin, a novel type I RIP from mature seeds of pumpkin (*Cucurbita moschata*) was tested in culture of human melanoma cells (Xia *et al.* 2003). Moschatin is a basic single-chain protein with a pI of 9.4 and MW of ≈ 29 kD. It displayed potent rRNA N-glycosidase activity and stronger inhibitory activity to protein synthesis than trichosanthin. Moschatin-Ng76 (the fraction containing immunotoxin) showed potent inhibition against melanoma cells M21 with IC₅₀ of 0.04 nM. Moschatin-Ng76 showed low cytotoxic activity on non-target Hela cells.

Acute and subacute toxicity

Toxicity in mice

Acute and subacute toxicity of a hydroalcoholic extract of *Cucurbita maxima* seeds was tested in female and male Swiss mice (Cruz *et al.* 2006). The extract was prepared by triturating air-dried seeds and macerating with ethanol 50% at room temperature for 15 days. The solvent was then removed under reduced pressure. Both acute (24 hours) and subacute (30 days) administration of the extract of the pumpkin seeds showed its low toxicity. The average lethal dose (LD₅₀) was higher than 5,000 mg/kg. Subacute treatment of 1,000 mg/kg b.w./day for 30 days resulted in an increase in body weight. Biochemical assays did not show any alterations in values of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and in haematological parameters (haemoglobin, red blood cell, hematocrit, and leukocyte) as compared to controls. However, observed

increased levels of the creatinine and urea can be related to adjustment of catabolism to high protein diet, so the urinary flow of the mice was considered normal. The macroscopic evaluation of organs (liver, spleen, heart lung and left kidney) did not differ from the control group.

Toxicity in rats and swine

Acute and subacute (30 days) toxicity of *Cucurbita maxima* Duch. seeds was studied in both rats and swine (Queiroz-Neto *et al.* 1994). Acute toxicity was tested in control and experimental groups of 10 Wistar rats. Subacute toxicity was tested in groups of 15 rats. Pumpkin seeds were prepared by triturating 10 g of seeds in 100 ml of water, filtered and administered by gavage at the dose of 1 ml/100 g b.w./day. Control groups received water. Tests were also performed in groups of 3 female swine (7-10 kg). The experimental group received pumpkin seed mixture with water prepared by triturating 10 g of seeds in a blender in 100 ml water and given orally to the swine at the dose of 10 mg/kg b.w./day. The control group received water. Both acute and subacute (30 days) administration of pumpkin seeds extract to the rats and swine did not induce any toxic effects as compared to controls. No changes in serum glucose urea, creatinine, total protein, uric acid, transaminases (GOT and GTP) alkaline phosphatase, lactic dehydrogenase and urea were recorded. Blood parameters were also not changed compared to control groups. The autopsy did not show any macroscopic abnormalities of heart, spleen and kidneys.

3.4. Overall conclusions on non-clinical data

Experimental non-clinical data presenting influence on urinary function, antiandrogenic, anti-inflammatory and antioxidant effects of pumpkin seeds and pumpkin seeds oil confirm the long tradition of their therapeutic use in prostatic hyperplasia and urinary tract disorders (irritable, overactive bladder).

Similar conclusions can be drawn from experiments performed with water, ethanol, methanol, butanol and ether extracts of *Cucurbita* seeds.

Moreover, cytotoxic, antifungal and antiparasitic effects were observed during investigations testing preparations containing the *Cucurbita* species *C. maxima* and *C. moschata*, which provide some theoretical background for their therapeutic use.

However, in some experiments the doses used were comparatively higher than the equivalent used in humans for medicinal purposes. For some experiments, information on the species was missing.

The published data on pharmacological activities support the traditional use of preparations containing pumpkin seeds in the proposed indications.

Studies on acute and subacute toxicity of *Cucurbita maxima* seeds in mice, rats and swine indicated only marginal or no effects in routine biochemical, haematological or macroscopical investigations.

No scientifically reliable studies were available about genotoxicity, carcinogenicity or reproductive toxicity.

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

Studies in benign prostate hypertrophy (BPH)

In a clinical study of Schilcher *et al.* (1987), 6 patients with BPH on 4th and 3rd day before surgery received orally 90 mg of a mixture of five Δ^7 -sterols daily. Δ^7 -sterols were isolated from the pumpkin seeds (*Cucurbita pepo* L. convar. *citrullina* var. *styriaca*). The control group did not receive any treatment. Mean serum levels of the prostate specific antigen and acid phosphatase were found significantly decreased one day after sterol administration compared to the pretreatment levels ($p < 0.05$). Unbound testosterone serum levels were significantly increased after 72 hours ($p < 0.05$), however total testosterone and sexual hormone binding globulin were not changed. Observations performed after surgery with excised tissue showed significantly lower levels of dihydrotestosterone in prostatic tissue of patients treated with Δ^7 -sterols compared to the control untreated group.

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

There are no data available on human pharmacokinetics.

4.2. Clinical Efficacy

4.2.1. Dose response studies

There are no specific data available on dose-response studies.

4.2.2. Clinical studies (case studies and clinical trials)

Clinical prospective studies with *Cucurbita pepo* products and with combination products are presented in Table 11 and Table 12.

Table 11. Clinical studies with *Cucurbita pepo* products

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
Friedrich <i>et al.</i> 2000	Open, multicenter	Inclusion criteria: BPH stadium I and II according to Alken <ul style="list-style-type: none"> ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume International Prostate Symptom Score (I-PSS) Quality of life questionnaire (LQ Index)	1) Prosta Fink Forte® capsule (500 mg of pumpkin seeds extract, 15-25:1 ethanol 92%) 1 – 2 capsules/day (equivalent to 10 g pumpkin seeds/day) 12 weeks	N=2245 1) 976 2) 1159 20 – 90 years; Mean: 64.8± years 85% of patients in age of 50- 80 years χ^2 Test	Mild gastrointestinal side effects (n=11), allergic skin reaction (n=1). In 96% of patients no side effects were registered	Significant decrease of I-PSS (41.4%) Significant improvement of quality of life (by 46.1%) <ul style="list-style-type: none"> ●Urinary day flow rates decreased from 5.8 to 2.1 (p<0.05) ●Nocturia decreased from 2.1 to 1.5 (p<0.05) 	Administration of pumpkin seeds extract for 12 weeks facilitates and efficient improvement of BPH symptoms, especially in early stages (Alken I and II)
Bach 2000	Controlled multicenter: (65centres), double blind	Inclusion criteria: BPH stadium I and II according to Alken	1) Prosta Fink Forte® capsule (500 mg of pumpkin seeds extract,	N=476 1) 233 2) 243	No differences between placebo and	1) I-PSS= 10.9 ±4.5	Administration of pumpkin seeds extract for 12 month induced

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
	with placebo group	<ul style="list-style-type: none"> ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume <p>International Prostate Symptom Score (I-PSS)</p> <p>1) I-PSS= 17.6±3.7</p> <p>2) Placebo group: I-PSS= 17.7± 3.8</p> <p>Quality of life questionnaire (LQ Index)</p>	<p>15-25: 1 ethanol (92%)</p> <p>1 – 2 capsules/day (equivalent to 10 g pumpkin seeds/day)</p> <p>2) Placebo Macrogol 400, glycerol, gelatine, E₁₇₂</p> <p>12 months</p>	<p>Mean: 63 years</p> <p>T-test</p>	<p>verum group were registered</p>	<p>change: -6.7 (-38%)</p> <p>Statistically significant difference between placebo and verum group</p> <p>2) Placebo group I-PSS= 12.2± 5.1 change: -5.5 (-31%)</p>	<p>significant reduction of I-PPS score in early stages (Alken I and II)</p> <p>Data of Qmax and prostate volume were not available. Changes of post-void residual volume and quality of life were identical under placebo and the plant extract</p>

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
Schiebel-Schlosser and Friedrich 1998	Open study	<p>Inclusion criteria: BPH stadium I and early II according to Alken</p> <ul style="list-style-type: none"> ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume <p>International Prostate Symptom Score (I-PSS):</p> <p>I-PSS=18.64</p> <p>Quality of life questionnaire (LQ Index):</p> <p>1.81</p>	<p>1) Prosta Fink Forte® capsule (500 mg of pumpkin seeds extract, 15-25:1 ethanol 92% w/w)</p> <p>1 – 2 capsules/day (equivalent to 10 g pumpkin seeds/day)</p> <p>3 months</p>	<p>Age: 20 – 90 years</p> <p>85% of patients in the age of 50 -80 years</p> <p>N=2245</p> <p>T-test</p>	<p>Side effects: gastrointestinal complaints, hypotension, allergy</p> <p>No side effects were registered in 96% of patients</p> <p>χ^2 Test</p>	<p>1) I-PSS= 10.94 change: 41.4% Statistically significant effects of treatment</p> <p>Quality of life (LQ index): 3.36 improvement (46.1%)</p>	Administration of pumpkin seeds extract for 3 month resulted in significant reduction of I-PPS score in early stages (Alken I and II) and improved quality of life

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
Hamvas <i>et al.</i> 1991	Open study	Inclusion criteria: BPH stadium I and II according to Alken <ul style="list-style-type: none"> ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume 	Verum: Pumpkin seeds oil – (Peponen [®] Capsule) 3 capsules 2 times/day for 1 month, later 3 capsules/day No control group 4 – 10 months	N=60 1) 26 10 months therapy 2) 22 7 months therapy 3) 12 4 months therapy χ^2 Test	No data	Administration of pumpkin seeds oil decreased symptoms of BPH in early stages (Alken I and II): decreased urinary flow rates, decreased of frequency of nocturnal urination and reduced feeling of difficult and painful discharge.	In 80% of treated patients significant improvement was noted.
Auel 1962	Open study Presentation of 16 case studies in patients with BPH symptoms	Inclusion criteria: BPH symptoms: Increased <ul style="list-style-type: none"> ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume 	Pumpkin seeds granulate –(Kürbis-Granufink [®]) 1 – 2 teaspoons /day 18 months	N=16 Age: 67 – 92 years	No data	Administration of pumpkin seeds oil decreased symptoms of BPH	

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
Weinkamer 1964	Open study Presentation of case studies from neuro-geriatric clinic in 40 male patients with BPH symptoms and in 40 female with bladder incontinence symptoms	Inclusion criteria: BPH symptoms: Increased ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume	Pumpkin seeds granulate ¹ –(Kürbis-Granufink®) 1 – 3 coffee spoons /day After 1 – 2 months Daily dose reduced to 1 coffee spoon/day 1 year	N=80 1) 40 Male 2) 40 Female Age: no data	No data	Administration of pumpkin seeds oil decreased symptoms of BPH and reduced bladder incontinence symptoms in female patients	

¹ in 100 g = proteins 22 g, lipids 32 g, carboxyhydrates 38 g, phytosterols, vitamins (vit. E 20-30 mg%, amino acids, metals-Fe, Mn, Zn, Cu, Se, cucurbitacin); seeds of *Cucurbita pepo* L. convarietas *citrullina* I. Greb. var. *styriaca*

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
Nitsch-Fitz <i>et al.</i> 1979	Open study	Inclusion criteria: Increased: ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume Stage I according to Alken	Granulate ² of pumpkin seeds (Kürbis-Granufink®) Dose: 3 tablespoon/day Duration: 8 weeks	N=43 N= 39 at the end of the study Median age = 54 years Exclusions: 3 patients excluded after gastrointestinal complaints. No statistical data	No statistical data	Administration of <i>Cucurbita pepo</i> ground seeds for 8 weeks induced improvement of BPH symptoms in more than 80% of patients.	

² in 100 g = proteins 22 g, lipids 32 g, carbohydrates 38 g, phytosterols, vitamins (vit. E 20-30 mg%, amino acids, metals-Fe, Mn, Zn, Cu, Se, cucurbitacin), seeds of *Cucurbita pepo* L. convarietas *citrullina* I. Greb. var. *styriaca*

Table 12. Clinical studies with combination products

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
Carbin and Eliason 1989	Pilot study, double blind with placebo group	Inclusion criteria: Increased: ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume	1) Mixture of extract of <i>Serenoa repens</i> (80 mg) and <i>Cucurbita pepo</i> seeds (80 mg) (CURBICIN®) 3 tablets 3 times/day 2) Placebo 3 months	N=26 1) 13 2) 13 1) 52–80 years mean: 66.3 years; 2) 52-81 years mean: 64.5 years Student T-test	No side effects were registered	Decrease of frequency of nocturnal micturitions compared to placebo group (p<0.03)	Administration of <i>Serenoa repens</i> and <i>Cucurbita pepo</i> seeds extract for 3 months induce improvement of BPH symptoms.
Carbin <i>et al.</i> 1990	Multicenter (6), randomized, double blind study, with placebo group	Inclusion criteria: Increased: ●prostatic volume ●voiding time (s) ●diurnal	1) Mixture of extract of <i>Serenoa repens</i> (80 mg) and <i>Cucurbita pepo</i> seeds (80 mg) (CURBICIN®) 2 tablets	N=26 1) 13 2) 13 1) 52–80 years mean: 66.3	No side effects were registered	Decrease of frequency of nocturnal micturitions compared to placebo group (p<0.03)	Administration of <i>Serenoa repens</i> and <i>Cucurbita pepo</i> seeds extract for 3 months induced improvement of BPH symptoms.

Reference	Quality of the study	Indications Baseline conditions	Preparation Daily dose Mode of administration Duration of treatment	Patients Number (N) Age Statistics	Adverse Events Toxicity	Final results Efficacy	Comment
		frequency <ul style="list-style-type: none"> •nocturnal frequency •urinary flow rates (ml/s) •residual urinary volume<300 ml Subjective variables: <ul style="list-style-type: none"> • dysuria • patient's evaluation of therapy 	3 times/day 2) Placebo 3 months	years; 2) 52-81 years mean: 64.5 years Student T-test			Increase of urinary flow (3.0±4.0 ml/s, p<0.001; decrease of micturition time (2.4±3.6 s, p<0.01; decrease of residual volume (42.5±62.1 ml, p<0.01; decrease of diurnal frequency (1.0±1.5 , p<0.05; decrease of nocturnal frequency (0.6±0.7, p<0.01

Four studies submitted by the Hungarian National Drug Agency describe experiences with the use of pumpkin seeds oil in male patients with prostatic hypertrophy (Hungarian Observational Studies with pumpkin seed oil, 1988-1989). Patients (N= 182) received pumpkin seed oil for 1, 2 (two trials) and 10 months, respectively, at the dose of 600 mg, 3 times daily. The treatment resulted in reduction of symptoms of painful and frequent urination, dysuria, nocturia and increase of urinary flow in majority of patients. Both patients' and doctor's judgment of therapy was in prevalence good or very good. On the basis of published results of these clinical trials, the quality of the available studies cannot be evaluated. Statistical methods are not shown in all protocols, in most trials inclusion and exclusion criteria are not given and no comparators (control groups) included. There is no information about drop-out cases. No specific questionnaires on the quality of life are given.

Clinical studies in urolithiasis

In the open clinical study in a group of 20 boys of 2 – 7 years of age (70% with malnutrition), a mixture of fresh, peeled and roasted pumpkin seeds was administered for 4 days to provide 60 mg/kg phosphorus/day. Chemical analysis of the composition of the seeds showed high concentrations of phosphorus (975.8 mg/100 g). Pumpkin seeds lowered calcium-oxalate crystal occurrence and calcium levels in urine and increased phosphorus and potassium levels. The authors concluded that high phosphorus levels in urine provided by pumpkin seeds can be a factor in lowering the risk of oxalate urinary stones (Suphakarn *et al.* 1987).

In the study by Suphiphat *et al.* (1993), 10 adolescents (13 – 16 years of age) with urinary bladder stones disease were given pumpkin seeds snacks for two days providing a level of 1.2 g of phosphorus/day. A 100 g of pumpkin seeds snack provided 696 mg of phosphorus. Pumpkin seeds induced inhibition of crystal formation of calcium-oxalate stones and raised pyrophosphate and magnesium excretion in urine. Elevation of both pyrophosphate and magnesium would inhibit calcium oxalate precipitation in the urinary tract.

Anthelmintic studies

In an observational study, 89 patients with acute schistosomiasis (3 – 41 years) were included with typical symptoms of fever, anorexia, cough, sweating, abdominal pain, tender liver and splenomegaly, diarrhoea and headache after duration of fever mostly after 3 weeks (Hsüeh-Chang and Ming 1960). Of all the cases, 72 were male, 17 female and the majority of the patients (59.3%) were children below 14 years of age. Eosinophilia was present in 93.3% of cases. X-rays of chest showed infiltration in 90.3% cases. In all 89 patients, schistosomal infection was proved by the hatching method in stool (in 42 cases other intestinal parasites were also found: *Ascaris* in 37, *Ancylostoma* in 4 and *Trichuris* in 1 case). Patients received powdered pumpkin (*Cucurbita moschata*) seeds, from which 20% of oil was removed by grinding before pulverization. Pumpkin seeds powder (80 g) was administered for 1 month to adults three times daily. For children weighing above 25 kg, full adult dose was administered, 2/3 the adult dose for 20-25 kg and the half of the adult dose for those weighing less than 20 kg. The fever declined steadily within 1-10 days in 75.3% cases. The liver diminished in size with lessening of tenderness. At the end of the treatment the patient's body weight increased (mean of 2.4 kg). Three repeated stool examination gave negative results for presence of miracidia in 7 cases out of 31 checked (22.6%) compared to that of antimony potassium tartrate treatment where positive miracidia were found in follow-up stool examination in 33-54.5% cases.

Plotnikow *et al.* (1972) studied, in an open clinical study including 150 patients with a diagnosis of taeniasis, the effectiveness of a treatment with a preparation of pumpkin seeds (therapeutic dose of 5–10 g). The therapeutic effectiveness varied between 50–70%.

In an observational study, 32 adults (20 males and 12 females) and a 13 years old girl with taeniasis were treated by use of a combination of areca and pumpkin seeds (Chung and Ko 1976). Specified amounts of areca nuts (75-150 g) and pumpkin seeds (50-100 g) were suspended in 1.5 l of tap water

and boiled to evaporate to 250 ml. All 33 patients received 250 ml of the mixture extract, two hours later magnesium sulfate as laxative was given and stool samples were collected for examination. A total of 48 worms including 42 scolices were recovered from 29 cases. Side effects and abdominal pain appeared. However, these symptoms are related to parasymphomimetic activity of arecoline present in areca nuts.

In another observational study, Feng (1956) found reliable therapeutic results in 96 patients infected with *Taenia saginata* and 20 patients with *T. solium* after combined use of areca catechu and pumpkin seeds. In *T. saginata* infected patients the cure rate was 95.19%, while in *T. solium* infected patients only 40% were cured. In treatment singular doses of areca catechu: 60-125 g and 80-125 g of pumpkin seeds were used.

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

No information available.

4.3. Overall conclusions on clinical pharmacology and efficacy

There are no data available from controlled clinical studies except one by Bach (2000). After 12 months of treatment, only a significant change of the International Prostate Symptom Score (I-PSS) compared to placebo was observed. The changes of Qmax, quality of life, prostate volume and post-void residual volume in the verum group were not different from that in the placebo group. The I-PSS is a score to assess the frequency of lower urinary tract symptoms suggestive of BPH, but it does not present the advancement of bothersomeness related to LUTS. The results of the Bach study show only a small effect on LUTS, without changing the objective parameters.

However, the published literature shows that the benefits of pumpkin seeds treatment depend on the tonic influence on the bladder, sphincter relaxation and alleviation of micturition symptoms. Due to mild effect on LUTS, pumpkin seeds, as other products of plant origin, can be used in patients without significant obstruction of the prostate and low risk for disease progression (Madersbacher *et al.* 2007). The available data allow considering the medicinal use of pumpkin seeds as traditional.

5. Clinical Safety/Pharmacovigilance

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

There are no adverse effects reported from the Member States, however allergic reactions to Cucurbitaceae family should be considered.

5.2. Patient exposure

No data are available.

5.3. Adverse events and serious adverse events and deaths

Information from products marketed in Germany

Information for the WEU soft extract and seven TU preparations from Cucurbitae semen authorised in Germany on risks (adverse drug effects, literature):

- rare: gastrointestinal complaints (diarrhoea, nausea, vomiting)
- frequency not known: hypersensitivity reactions (urticaria, facial oedema, anaphylactic reaction/shock).

Information from clinical studies

In most publications from the open clinical studies of pumpkin seeds, there were no adverse effects registered. Only two studies, by Schiebel-Schlosser and Friedrich (1998) and Friedrich *et al.* (2000), reported mild gastrointestinal complaints in no more than 4% of the patients. It seems that upon administration within the therapeutic dose range, there is no risk of serious side effects.

Allergy

Allergy to *Cucurbita pepo* was detected in four patients complaining of pruritus, nausea and diarrhoea after oral intake. Specific serum IgE levels to *Cucurbita* were found in all cases (Reindl *et al.* 2000).

Allergy to pumpkin seed after ingestion was recorded in three patients with symptoms of itching, swelling of oral mucosa and asthma (Fritsch *et al.* 1997). The immunoblot revealed pumpkin seed allergens 14-kDa protein which presumably represents a homologue of the panallergen profilin.

The case of occupational rhinitis and asthma symptoms to *Cucurbita pepo* with detection of specific IgE with positive results of class 2 was recorded (Miralles *et al.* 2000).

Allergy to raw zucchini was also observed by Vieths *et al.* (2002), with profilin allergen in 2 of 4 cases.

A case of acute allergic reaction in a 2.5 years old boy with vomiting, coughing, dyspnoea, and urticaria after drinking two spoons of a vegetable soup prepared from *Cucurbita maxima* was described by Hagendorens *et al.* (2009). Upon challenge with the pumpkin, basophils of the patient up-regulated from 1% up to 66% for the highest stimulation concentration. Allergic reaction presented underlying IgE-mediated mechanism.

Figueredo *et al.* (2000) described the case of an acute allergic reaction in a 28-year-old woman who was allergic to pollens and cat dander 15 minutes after eating pumpkin soup. The patient suffered from generalized itching, angioedema of the lips and face and mild dyspnoea and required hospital treatment. The skin prick tests with fresh fruits were positive to pumpkin and also to other Cucurbitaceae fruits: cucumber, zucchini, muskmelon and watermelon. An IgE-mediated mechanism was suggested.

5.4. Laboratory findings

No data are available.

5.5. Safety in special populations and situations

Interactions

Yue and Jansson (2001) described two cases of coagulation disorders with increased INR values that were associated with the combination medicinal product (active constituents: extracts of *Serenoa repens*, *Cucurbita pepo* and vitamin E). The INR normalized after discontinuation of treatment.

However, pure vitamin E administration can be related to prolongation of coagulation time, therefore a causal relationship for pumpkin extract in this interaction could not be established.

Use in children and adolescents

No experimental studies are available.

The use in children and adolescents under 18 years of age is not recommended because lower urinary tract symptoms in these populations require medical supervision.

Fertility, pregnancy and lactation

No experimental studies are available.

The use in pregnant women is not recommended because lower urinary tract symptoms in this population require medical supervision.

No concern has arisen about any malformation in humans, following the consumption of pumpkin seeds and fatty oil. Can be used during pregnancy and lactation if clinically needed.

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

No data on fertility are available.

Overdose

None reported.

Effects on ability to drive and use machines

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

5.6. Overall conclusions on clinical safety

There are no data available from most clinical trials. The common use of pumpkin seeds as food proves not to be harmful. Some reported side effects concerning gastrointestinal reactions due to the pumpkin seeds preparations intake are acceptable.

Administration of pumpkin seed preparations can be regarded as safe and justified, when using therapeutic doses.

6. Overall conclusions

Seeds of *Cucurbita pepo* and herbal preparations thereof have been in medicinal use for at least 30 years with at least 15 years in the EU. The long-standing medicinal use as well pharmacological data make the use in the proposed indication plausible. There are no sufficient data from well-designed clinical trials to support a well-established use in this indication. Therefore the medicinal use of pumpkin seed and preparations thereof has to be regarded as traditional in the sense of Directive 2004/24/EC. The outcome of the clinical trials support the plausibility in the proposed indication.

In conclusion, pumpkin seeds can be used for the "relief of lower urinary tract symptoms related to benign prostatic hyperplasia or related to an overactive bladder, after serious conditions have been excluded by a medical doctor".

However pumpkin seeds can be only used in conditions which are controlled by a doctor with regular medical checks (Roehrborn 2008; Guideline on the management of benign prostatic hyperplasia (BPH) 2003 (revised 2010 Jan) NGC:008255. <http://guideline.gov/content.aspx?id=25635&search=bph>).

Administration of pumpkin seed preparations can be regarded as safe and justified, when administered in the specified conditions of use and at the specified therapeutic doses.

Due to the lack of data on genotoxicity, a Community list entry for pumpkin seeds cannot be established.

Annex

List of references