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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)

Draft

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 as such or herbal preparation in solid dosage form for oral use

Note: This draft Assessment Report is published to support the release for public consultation of the draft Community herbal monograph on *Cucurbita pepo* L., semen. It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.



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1. Introduction

Cucurbita pepo L. (pumpkin) belongs to the melon family *Cucurbitaceae* which comprises approximately 120 genera and 760 species.

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 (1994), Gruenwald *et al.* (ed). (2000; 2004), German Commission E Monograph (1991), Duke's Handbook of Medicinal Herbs (Duke 2002), 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 irritable bladder. The pumpkin seeds yield approximately 50% oil, (mostly linoleic and oleic acid and tocopherol), but the main active constituents are Δ^7 sterols (avenasterol, spinasterol) and Δ^5 sterol (sitosterol, stigmasterol). The advantage of pumpkin seeds treatment arises from its tonic influence on the bladder and sphincter relaxation. A *Cucurbita pepo* treatment alleviates micturition symptoms but does not decrease the augmented volume of prostate gland.

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. *citullina* 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 family of *Cucurbitaceae* are popular for light meals. They are categorised to *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita mixta* and *Cucurbita moschata* as to the pattern and the structure of their branches.

Constituents of the pumpkin seeds

Older literature 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). Data on the constituents of the seeds from *Cucurbita pepo* L. convar. *citullinina* var. *styriaca* have been summarised by Schilcher (1986). 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, the main component of the seeds, and its content of fatty acids, tocopherols, carotenoids, chlorophyll pigments, squalene, and sterols are available 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%) (Schuster *et al.* 1983, Murkovic *et al.* 1996; Tsaknis *et al.* 1997).

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 color 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 color (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).

The composition of fatty acids varies depending on several factors: variety of areas in which the plants are grown, climate, state of ripeness. The variability in the oil content is very high resulting from a broad genetic diversity (Murkovic *et al.* 1996a).

The glyceride fraction contains over 80% unsaturated fatty acids, mainly linoleic (C18:2; 42-64%) 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%) (Andrikopoulos *et al.* 2004, Schilcher 1986; Murkovic *et al.* 1996a; 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. 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 a trace amounts of α - and γ - linolenic acids were found (Glew *et al.* 2006).

Table 1. Fatty acids composition of pumpkin seed kernels (%) (Alfawaz 2004)

Fatty acid	Mean value (%)
Myristic (C _{14:0})	0.18±0.03
Palmitic (C _{16:0})	16.41±0.95
Stearic (C _{18:0})	11.14±1.03
Palmitoleic (C _{16:1})	0.16±0.04
Oleic (C _{18:1})	18.14±0.60
Erucic (C _{22:1})	0.76±0.13
Linoleic (C _{18:2})	52.69±0.92
Linolenic (C _{18:3})	1.27±0.22
Total saturated	27.73±1.8
Total unsaturated	73.03±0.78
Monounsaturated	19.06±0.49
Polyunsaturated	53.97±1.15

n=12 sample, in duplicate

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).

The dominant fatty acids that are found in pumpkin seed oil are palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2). 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).

The content of unsaponifiable fraction

Sterols, up to 0.5% of the oil (55-60% of the unsaponifiable fraction), predominantly Δ^7 sterols, which are considered to be the key active constituents of pumpkin seed in the treatment of benign prostatic hyperplasia (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; Schilcher 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 - 3β - ol), $\Delta^{7,22}$ - stigmastadienol = α -spinasterol (stigmasta - 7,22- dien- 3β - ol), $\Delta^{7,22,25}$ - stigmastatrienol (stigmasta - 7, 22, 25-trien- 3β - ol), Δ^7 - ergostenol (27-methylcholest-7-en- 3β - ol) and $\Delta^{7,24,28}$ -stigmastadienol = Δ^7 - avenasterol (stigmasta-7, 24 (28) - dien- 3β -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- 3β - ol (Bombardelli and Morazzoni 1997); 5α - stigmasta-7,25-dien- 3β - ol (Sauter *et al.* 1985) and 24β - ethyl - 5α - cholesta-7,25 (27)-dien- 3β -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- 3β - ol, α -spinasterol, $\Delta^{7,25}$ -stigmastadienol and Δ^7 - avenasterol, contain also: stigmasterol (stigmasta-5,22-dien- 3β -ol), 24-methylcholest-7-enol and Δ^7 - stigmastenol (stigmasta-7-en- 3β - ol), and trace amounts of cholesterol (cholest-5-en - 3β - ol), brassicasterol (ergosta-2,22-dien- 3β -ol), campesterol (campest-5-en- 3β -ol), sitostanol (stigmastan- 3β -ol), Δ^5 - avenasterol (stigma-5,24(28)-dien- 3β -ol), erythrodiol, and uvaol (Tsaknis *et al.* 1997).

In the seeds of *C. pepo* L. convar. *citrullinina* Greb. var. *styriaca* Greb 24-ethyl- Δ^7 -steryl glucosides were identified as: 3-O-(β -D- glucopyranosyl) - 24β -ethyl- 5α -cholesta-7,25 (27)-dien- 3β -ol, 3-O-(β -D- glucopyranosyl) - 24β -ethyl- 5α -cholesta-7, trans-22, 25 (27)-trien- 3β - ol, 3-O-(β -D- glucopyranosyl) 24α -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; Schilcher 1986).

Triterpenoids: including relatively large amounts (0.2-0.08%) 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 presence of cucurbitacins in seeds can not 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).

- **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 (Schilcher 1986; Sauter *et al.* 1985; Bombardelli and Morazzoni 1997).

- **Tocopherols,**

ca. 360-540 mg/kg of oil, comprise β - and γ - (but not α -) tocopherols (Vogel 1978; Schilcher 1986). Pumpkin seeds were found to have the greatest content of tocopherols (16 mg/100g) 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). This finding was confirmed by Lazos (73 mg/kg) (Tsaknis *et al.* 1997).

Other report showed an average of 338 mg/kg of β - + γ - tocopherol 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 $\mu\text{g/g}$, one of the highest values found in plants (Bombardelli and Morazzoni 1997; Kreft *et al.* 2002). Other sources

are reporting even higher amount of selenium: 1.29 µg/g (Glew *et al.* 2006). Mean estimated maximum intake: 0.4 mg/day and safe upper level was estimated for 0.45 mg total selenium/day for daily consumption over a lifetime (Scientific Opinion 2009).

The pumpkin seeds contained relatively large amounts of potassium (5790 µg/g dry weight) and chromium (approx.3 µg/g dry weight). However, the sodium content of pumpkin seeds was low (6.9 µg/g dry weight). Pumpkin seeds contained relatively large amounts of magnesium Mg (5690); zinc Zn (113); copper Cu (15.4); molybdenum Mo (0.805) and another minerals: phosphorus P (15700); calcium Ca (346); iron Fe (106); manganese Mn (49.3); aluminum Al (9.21); barium (1.16); cobalt Co (0.29); strontium Sr (1.83); nickel Ni (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 955.81mg, and iron 8.06 mg.

Pumpkin seed of *Cucurbita pepo* Kakai 35 are rich in minerals: P 17.831; K 13.736; Mg 5.688; Ca 1.643; Fe 0.211; Na 0.332; Cu 0.016; Zn 0.190; Mn 0.080 (in g kg⁻¹) (Mansour *et al.* 1993).

- **Proteins and amino acids**

Proteins are abundantly present in the seeds, with values that spread between 31% and 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 Mansour *et al.* (1993) the dehulled and defatted pumpkin seed (*Cucurbita pepo* Kakai 35), has an excellent pattern of amino acids, contain high levels of most essential amino acids including: isoleucine 2.66; leucine 6.13; lysine 5.20; cystine 1.52; methionine 1.25 ; tyrosine 2.94; phenylalanine 4.00; threonine 2.75; tryptophan 1.56; valine 3.40; histidine 3.62; and non- essential amino acids: arginine 16.70; aspartic acid 10.19; glutamic acid 18.13; serine 5.46; proline 4.34; glycine 5.86; alanine 4.29 (in g per 16 g N).

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-carboxypyrrolidine for its anthelmintic properties isolated from *Cucurbita pepo* (0.18-0.66%) and *Cucurbita moschata* Duch, (0.4-0.84%) seeds. Cucurbitin 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, Rybałtowski 1966).

Moschatin, a novel type I ribosome-inactivating protein (RIP) from mature seeds of pumpkin (*Cucurbita moschata*) and from sacrocarp of *Cucurbita pepo* and *moschata* were found (Barbieri *et al.* 2006). RIPs are considered to be plant defense-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 RIP's in edible vegetables due to their antibacterial and antiviral activity could even be beneficial.

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

The abumin with ribonuclease (RNase) activity (2S albumin) was purified from pumpkin seeds (*Cucurbita sp.*). It 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 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). From the seeds of *C. pepo* and other *Cucurbitaceae*, some low molecular weight 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 knottin structure. The level of antitrypsin activity in the seeds of some Cucurbitaceae plants varied between 31 and 399 units/100 g of fresh tissue (Bombardelli and Morazzoni 1997).

Inhibitors of trypsin and activated Hageman factor (a serine protease implicated in blood coagulation) 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. Trypsin inhibitors activity in *Cucurbita pepo* and *Cucurbita moschata* seeds was low as compared to *Glycine max* activity (Henderson *et al.* 1986).

Peponin, a single-chain, ribosome-inactivating protein 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 low level of total carbohydrate (91.3 g/kg), 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⁻¹) (Mansour *et al.* 1993). A proximate analysis of 100g roasted pumpkin seeds: vitamin A 3.32 μ g, thiamin 0.28mg, riboflavin 0.05mg, vitamin C 30.38mg, 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 \rightarrow 2) - β - D- glucopyranoside, 4 - (2 - hydroxyethyl) phenyl 5-O- (3-methylbutyryl)- β -D-apiofuranosyl(1 \rightarrow 2)- β -D-glucopyranoside, 4-(2-hydroxyethyl) phenyl 5-O-nicotinyl- β -D-apiofuranosyl(1 \rightarrow 2)- β -D-glucopyranoside, 4-(2-hydroxyethyl) phenyl 5-O-(4-aminobenzoyl)- β -D-apiofuranosyl (1 \rightarrow 2)- β -D-glucopyranoside, 4-(2-hydroxyethyl)-2-methoxyphenyl 5-O-(2-S-2-methylbutyryl)- β -D-apiofuranosyl (1 \rightarrow 2)- β -D-glucopyranoside, 4-(hydroxymethyl) phenyl 5-O-(2-S-2-

methylbutyryl)- β -D-apiofuranosyl (1 \rightarrow 2)- β -D-glucopyranoside, 4-(hydroxymethyl) phenyl 5-O-nicotinyl- β -D-apiofuranosyl (1 \rightarrow 2)- β -D-glucopyranoside, and 4-(hydroxymethyl) phenyl 5-O-(4-aminobenzoyl)- β -D-apiofuranosyl (1 \rightarrow 2)- β -D-glucopyranoside, on the basis of various spectroscopic analyses and analyses of hydrolysis product.

The structures are closely related to the previously isolated cucurbitosides A-E from the seeds of *C. moschata* all these compounds contain the 5-O-acyl- β -D-apiofuranosyl (1 \rightarrow 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; cucurbitic 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).

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

- Herbal substance(s)

Whole, ripe and dried seeds

- 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 product
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 product
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
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 supplement
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:	
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 supplement
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 supplement
Portugal	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No product
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 product
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:	Food supplement
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No product
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.

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 Hippocrates, 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 thirty years in the Community, 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; PDR 2000; 2004).

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

- **Austria**

Is the Herbal Substance on the market? Yes No

Traditional Use

- Preparations (kind of extract, extraction solvent, DER)

1) Combination product (capsules)

1 capsule contains:

Cucurbita seed 400 mg

Cucurbita seed oil 340 mg

Dry extract of *Serenoa repens*, fruit, DER 7-13:1, extraction solvent ethanol 90% (m/m) 75.0 mg

2) Combination product

1 capsule contains:

Cucurbita seed 400 mg
Cucurbita seed oil 340 mg

- Since when are the preparations on the market?

- 1) 2008
- 2) 2008

- Pharmaceutical form (Standard Terms)

- 1) Capsule
- 2) Capsule

- Posology (Route of administration in Standard Terms + daily dosage)

- 1) Adults: 1 capsule 3x daily
- 2) Adults: 3-5 capsules daily

- Indications

- 1) Dysfunctions of the urinary tract in men
- 2) Dysfunction of the bladder and for facilitation of urination

- Risks (adverse drug effects, literature)

No data

Is the Herbal Substance on the market? Yes No

Status Authorised products Registered products Food supplements

Combination products

The herbal substance is only available in combination products.

Average number of combination substances: 2-3 3-5 >5

• Germany

Is the Herbal Substance on the market? Yes No

- Since when are the preparations on the market?

At least since 1976

Well-Established Use

- Preparations (kind of extract, extraction solvent, DER)

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

- Pharmaceutical form (Standard Terms)

capsule, soft

- Posology (Route of administration in Standard Terms + daily dosage)

For oral use in adults, 2 x daily 1 capsule containing 500 mg dry extract

- Indications

For relief of lower urinary tract symptoms related to benign prostatic hyperplasia (Alken stages I to II or Vahlensieck stages II to III).

- Risks (adverse drug effects, literature)

Rare: gastrointestinal complaints (diarrhoea, nausea, vomiting)

Frequency not known: Hypersensitivity reactions (urticaria, facial oedema, anaphylactic reaction/shock)

Is the Herbal Substance on the market? Yes No

- Status

Authorised products Registered products Food supplements

- Were pharmacovigilance actions taken on medicinal products containing the herbal substance?

Yes No

What are the main combination substances?

Combination products

The herbal substance is only available in combination products.

Average number of combination substances: 2-3 3-5 >5

What are the main combination substances?

Other information on relevant combination products:

In Germany there are no authorised combination products.

Traditional Use*

- Preparations (kind of extract, extraction solvent, DER)

1, 5, 6) Cucurbitae oleum

2, 7) dry extract (15-30:1) from Cucurbitae semen, extraction solvent: ethanol 60% v/v

3) Cucurbitae semen

4) Cucurbitae semen

(*Cucurbita pepo* L. *convar. citrullinina* I. GREB. *var. styriaca* I. GREB.)

- Since when are the preparations on the market?

1-7) at least since 1976

- Pharmaceutical form (Standard Terms)

1, 5, 6) capsule, soft

2) capsule, hard

3) granules

4) herbal substance

7) film-coated -tablet

- Posology (Route of administration in Standard Terms + daily dosage)

All for oral use in adults and adolescents over 12 years

- 1) daily 3-4 containing 1000 mg Cucurbitae oleum each
- 2) 3 x daily 1 containing 105 mg dry extract
- 3) adults: 8-23 g granules daily; adolescents over 12 years: 3-6 g granules daily (100 g granules contain 66 g Cucurbitae semen)
- 4) 5-15 g daily divided in 2 doses (one in the morning and one in the evening)
- 5, 6) 3 x daily 2 containing 583 mg Cucurbitae oleum each (corresponding to 1166 mg Cucurbitae oleum per single dose and 3498 mg Cucurbitae oleum per day)
- 7) 2 x daily 1 containing 152 mg dry extract

- Indications

Traditionally used to strengthen bladder function

- Risks (adverse drug effects, literature)

Rare: gastrointestinal complaints

Frequency not known: Hypersensitivity reactions (urticaria, facial oedema, anaphylactic reaction/shock)

Is the Herbal Substance on the market? Yes No

- Status

Authorised products Registered products Food supplements

- Were pharmacovigilance actions taken on medicinal products containing the herbal substance?

Yes No

Combination products

The herbal substance is only available in combination products.

Average number of combination substances: 2-3 3-5 >5

- What are the main combination substances?

Cucurbitae oleum

Sabalís serrulatae fructus

Other information on relevant combination products:

In Germany there are 10 authorised combination products.

Number of combination substances	Number of authorized 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

**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.*

- **Poland**

Traditional Use

- Preparations (kind of extract, extraction solvent, DER)

1) Cucurbitae peponis oleum virginum

- Since when are the preparations on the market?

1) 1990

- Pharmaceutical form (Standard Terms)

1) Soft capsules

- Posology (Route of administration in Standard Terms + daily dosage)

1) 2 x daily 2 capsules, before meals, during 4 weeks.

- Indications

1) Traditionally in troubles with urination caused by benign prostatic hyperplasia.

- Risks (adverse drug effects, literature)

Not recommended in hypersensitivity for any of product components.

It is recommended to regularly perform urological examinations

Is the Herbal Substance on the market? Yes No

- Status

Authorised products Registered products Food supplements

- Were pharmacovigilance actions taken on medicinal products containing the herbal substance?

Yes No

Combination products

The herbal substance is only available in combination products.

Average number of combination substances: 2-3 3-5 >5

What are the main combination substances?

Other information on relevant combination products

Additional comments:

There are 3 similar food supplements (similar posology, composition, pharmaceutical form), containing pumpkin oil, on Polish market.

- **Spain**

There are no authorised or registered herbal medicinal products of *Cucurbita pepo* in Spain.

Additional comments:

There are some products on the market sold as food. *Cucurbita pepo* seed oil in soft capsules, from 200 mg to 1000 mg. Daily dose: 1-2 g/daily. It is also possible to find fresh seeds. Toast seeds are a common food.

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

British Herbal Compendium (Bradley, 2006)

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.

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. Pumpkin seed (*Cucurbitae peponis semen*). Published 30.11.1985, revised 17.01.1991

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

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: no information

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)

Indications: 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.

Daily oral 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)

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

Dosage: pumpkin seeds, 1 – 2 heaped table spoonfuls (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)

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

Dosage: Ground or chewed pumpkin seeds, 1 – 2 heaped table spoonfuls (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)

Indications: anthelmintic (taenifugae) and benign prostatic hyperplasia.

Dosage: As taenifugium 30 – 60 -150 g, after 2 hours laxative intake. In symptoms associated with enlarged prostate 1 teaspoon of seeds 3 times daily. Maximal dose is not described.

Duration of use: no information

Martindale 2009

Seeds of *Cucurbita pepo* were formerly used for the expulsion of tapeworms. It is ingredient of several herbal preparations used in urinary-tract disorders.

Dose: no information

Duration of use: no information

PDR for Herbal Medicines (2000)

The medicinal parts are the fresh and dried seeds.

Indicated in irritable bladder and prostate complaints. This medication relieves only the symptoms associated with an enlarged prostate without reducing the enlargement.

Daily oral 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)

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.

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

Duration of use: no information

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 commercially available pumpkin seeds (*Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita mixta*) extracts were screened for their antioxidant activity and their inhibitory activity

against lipid peroxidation (Xanthopoulou *et al.* 2009). 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.

Antiandrogenic activity

An ethanolic extract of *Cucurbita pepo* did not show antiandrogenic activity. Experiments were performed *in vitro* by use of an androgen receptor responsive reporter gene assay (Schleich *et al.* 2006). For the extract preparation, 15 g of pumpkin seeds were crushed in a mortar. The extract was produced with 500 ml of ethanol and filtered through 0.7 µm filter paper, the evaporated under vacuum at 40°C yielding 7.03 g dry mass. The ethanolic extract of *C. pepo* contained mainly triglycerides, Δ⁵ – and Δ⁷ sterols and tocopherol.

Schmidlin and Kreuter (2003) described influence of the *Cucurbitae pepo* extract on 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 experimental model of of the prostatate hyperthrophy induced by injection of testosterone in Sprague-Dawley castrated male rats. The incubation human placenta homogenate with 10 mg/ml of the extract induced 44.7±0.2 – 55.9±15.7% reduction of activity of aromatase. Activity of the enzyme 5-α-reductase Typ II in human placenta homogenate was inhibited at 90.4±2.4%, 71.4±4.1% and 29.6±7.9% respectively by 10 mg/ml, 2 mg/ml and 0.4 mg/ml concentrations of the extract. Activity of the enzyme 5-α-reductase Typ II in rat placenta homogenate was inhibited at 69.7±2.8%, 27.0±1.5.1% and 6.5±1.1% respectively by 10 mg/ml, 2 mg/ml and 0.4 mg/ml concentrations of the extract. Activity of the enzyme 5-α-reductase Typ II in human embryonic kidney (HEK 293) cells was inhibited at 52.0±2.4%, 28.0±2.4.1% and 14.8±7.8% respectively by 50 mg/ml, 20 mg/ml and 5 mg/ml concentrations of the extract. Testosterone (1mg/kg) injection in castrated rats after 4 days induced an increase of weight of prostate gland to 41±1.9 mg/100g kg from 13.7±1.9 mg/100g kg (100% arbitrary reduction). After subcutaneous finasterid injection (1 mg/kg) in positive control group reduction of the weight of prostate glands was 76% (p<0.01, n=7) and in pumpkin extract group (100 mg/kg, for 4 days, n=7) the decrease of weight was 31%.

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* (*citrullinina* GREB. var. *styriaca* GREB) from biological cultivation were finely grounded (10 g of the powder was added to 100 ml of medium Roswell Park Memorial Institute (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 capsules (1 capsule – 0.72 g was added to 30 ml supplemented RPMI and mixed); the highest concentration reached was 38.4 µg/ml seeds from capsule. All extract were sterile filtered at 0.2µm sieve.

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 coincubation 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 µg/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. A significant decrease was evident after incubation of 1mg/ml ($p<0.05$), 10 mg/ml ($p<0.01$) and 50 mg/ml ($p<0.001$) of the cold, shaken for 10 minutes water extract; 10 mg/ml ($p<0.001$) and 50 mg/ml ($p<0.001$) of the hot extract and after 12 mg/ml (0.001) of capsules of pumpkin seeds extract compared to controls.

An increase of concentration of tryptophan in PMBC stimulated with phytohaemagglutinin as a control was registered. Significant changes were noted after coincubation (48 hours) with a cold extract of 10 mg/ml ($p<0.01$) and 50 mg/ml ($p<0.01$), hot extract of 10 mg/ml ($p<0.01$) a 50 mg/ml ($p<0.01$) and 12 mg/ml ($p<0.01$) of the extract of capsules compared to control.

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) (Gerhäuser *et al.* 1993). 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 induced inhibition of cell-free protein synthesis ($ID_{50}=5.4\pm 1.0$ ng/ml, $n=4$) was found to be strong inhibitor of HIV-1 RT ($ID_{50}=12.7\pm 0.31$ μ g/ml, $n=2$).

Antifungal activity

The cucurmoschin, 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). 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.

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).

Different basic polypeptides isolated from the soluble and cell wall-derived fractions of *Cucurbita maxima* seeds (Vassiliou *et al.* 1998) using HPLC methods. Fractions containing the major 2249, 4650 and 11696 Da components exhibit antifungal activity. The antifungal activity of proteins from *Cucurbita maxima* seeds was determined using growth curves after 60 h of incubation using fixed protein concentration (Table 2).

Table 2. 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)	(51%, 59)
<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)

(IC₅₀ values) = protein concentrations required for 50% inhibition of growth after about 60 h of incubation

AFP = antifungal proteins

Protein concentrations required for 50% inhibition of growth after about 60 h of incubation (IC₅₀ 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 h is given in brackets, together with the concentration of antifungal protein preparation used, in µg/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. AFP – antifungal proteins.

In vivo experiments

Influence on urinary functions

The effects of non-specified 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 3. and Table 4.) (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 3. 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 4. 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*
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, weight of prostate size and improved histology of testis. Pumpkin seeds given orally (2.5, 5 and 10% in a diet) dose dependently inhibited enlarged 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/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. Seed oil (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/body weight of the rat) was established. Testosterone significantly increased prostate size ratio (p<0.05) and this increase was significantly inhibited by treatment of pumpkin seed oil at 4.0 mg/100 g body weight.

Tsai *et al.* (2006): 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) tested pumpkin seed oil efficacy for 14 days. 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).

Assessor's 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

In experiment performed on groups of 4 anesthetized rabbits, the urethral and bladder pressure were determined 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 min after injection. Statistically significant

($p < 0.05$) decrease of bladder and urethral pressure was registered only after oil fraction injections. After increasing the number of rabbits in oil preparation group for 8, the decrease of bladder pressure was 7.6 mm Hg ($p < 0.001$) with maximum effect after 2 hours, and urethral pressure 5.4 mm Hg ($p < 0.01$) as compared to pre-treatment values. The other: n-butanol and ether fraction had no significant influence (Zhang *et al.* 1994).

Cardiovascular activity

The ethanolic extract from *Cucurbita maxima* dried powdered seeds was extracted with 80% of ethanol, the filtrate was evaporated and the residue (PE) was dissolved in distilled water (concentration 500 mg/ml). Ethanolic extract induced 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).

Antiinflammatory 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 CCl_4 intoxication in low-protein fed Sprague-Dawley rats (Sprague Dawley, 80-90 g bw) (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 IV injection of CCl_4 . Two hours after the CCl_4 administration, one group of rats received 1 ml/kg body weight of the pumpkin seed protein isolate in saline (20 g/100 ml) by gavage. Autopsy was performed at 2, 24, 48 and 72 h after the CCl_4 intoxication and plasma samples and liver tissue were tested. CCl_4 intoxication resulted in increase in lipid peroxydation (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 CCl_4 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 h 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 total antioxidant capacity (TAC) was significantly increased at all intervals tested ($p < 0.05$). Glucose-6-phosphatase 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 bw). 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 body weight. The control group received 10 ml/kg polyethylene glycol 400 (PEG 400: physiological saline (1:1)/kg body weight. Two hours after the acetaminophen administration, one group of rats received 1ml/kg body weight of the pumpkin seed protein isolate in saline (20g/100 ml) by gavage. Autopsy was performed at 2, 24, 48 and 72 h 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 body weight) 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 h 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 of whole, triacylglycerides in group receiving sifted pumpkin flour ($p < 0.05$) (Table 5).

Table 5. 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. Three groups of rats received control (CD) nutrient diet for rodents, the second group received CD-chol diet supplemented with 1% of cholesterol and the third MS-chol group CD diet supplemented with seed mixture powder substituted at 33% and with 1% of cholesterol. One month's treatment resulted in MS-chol group significant decrease of lipid parameters compared to CD-chol group (Table 6). 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 6. 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**	10.67±0.38 ⁺⁺
<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: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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

In an estrogen 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 7).

Table 7. 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 – Triglycerides

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 body weight of the pumpkin seed protein isolate in saline (20 g/100 ml) by gavage. Autopsy was performed at 2, 24, 48 and 72 h after the CCl₄ intoxication and plasma samples were tested. The significant increased 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

The anticestodal activity of the *Cucurbita maxima* seed powder for 10 days (50, 100 and 200 mg/kg of feed) tested *in vivo* in domestic fowl did not result in any significant anthelmintic activity; only in 8.69% a removal of *Raillietina cesticii* cestodes after feeding the birds with 2 ml of ethanolic (80%) extract (300 mg/ml) (Lahon *et al.* 1978).

Aminoacid 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 administered orally to mice at the dose 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 worm 26.4, 25.3, 18.0, 11.8, 8.8 respectively while that of the control infected group was 34.0 (p<005). 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 5000 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 8).

Table 8. Nematicidal effect of powdered pumpkin seeds in piglet compared to ivermectin 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)

Marie-Magdeleine *et al.* (2009) tested *in vitro* activity of three extracts of *Cucurbita moschata* seeds on four developmental stages of development of parasite of small ruminants nematode *Haemonchus contortus*. From the three extracts (aqueous, methanolic 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).

Mahmoud *et al.* (2002) described therapy of experimental infection of 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.

Cucurbitine data: Pharmacokinetics was tested in male mice after oral or intraperitoneal administration of ¹⁴C 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]labeled cucurbitine showed that radioactivity was highly localized in the liver, kidney, dorsal root ganglion, tracheal cartilage and pancreas at 20 min after *iv* 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 on 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

Inhibition by pumpkin seed extract of protein synthesis in the rabbit reticulocyte lysate was measured (Gasperi-Campani *et al.* 1977). The concentration of the extract which inhibited protein synthesis was 50% (ID₅₀=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 h, then centrifuged at 20 000 x for 20 min 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 ribosome-inactivating protein 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 molecular weight of ≈29kD. 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 the 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 h) and subacute (30 days) administration of the extract of the pumpkin seeds showed its low toxicity. The average lethal dose (LD₅₀) was higher than 5000 mg/kg. Subacute treatment of 1000 mg/kg/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 (hemoglobin, 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 body weight/rat/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/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 preclinical data presenting influence on urinary function, antiandrogenic, antiinflammatory and antioxidant effects of pumpkin seeds and pumpkin seeds oil confirm long tradition of their therapeutic use in prostatic hyperplasia and urinary tract disorders (irritable 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 when testing other *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.

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 hyperthrophy (BPH)

In clinical study of Schilcher *et al.* (1987), 6 patients with BPH on 4th and 3rd day before surgery received 90 mg of a mixture of five delta-7-sterols daily by oral way. Delta -7-sterols were isolated from the pumpkin seeds (*Cucurbita pepo* L. convar *citrullinina* 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 delta-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* and combination products are presented in Table 9 and Table 10.

Table 9. 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 with placebo group	Inclusion criteria: 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%) •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 with placebo	Inclusion criteria: Stadium I and II according to Alken <ul style="list-style-type: none"> •prostatic volume •nocturia 	1) Prosta Fink Forte capsule (500 mg of pumpkin seeds extract – 15-25:1 ethanol 92%)	N=476 1) 233 2) 243 Mean:	No side effects were registered	1) I-PSS= 10.9 ±4.5 (change: -6.7 (-25.4%))	Administration of pumpkin seeds extract for 12 month induced significant

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
	group	<ul style="list-style-type: none"> urinary flow rates residual urinary volume International Prostate Symptom Score (I-PSS) 1) I-PSS=17.6 ±3.7 2) Placebo group: I-PSS=17.7± 3.8 Quality of life questionnaire (LQ Index)	1 – 2 capsules/day (equivalent to 10g pumpkin seeds/day) 2) Placebo Macrogol 400, glycerol, gelatine, E ₁₇₂ 12 months	63 years T-test		Statistical significant difference between placebo and verum group 2) Placebo group I-PSS=12.2± 5.1 (change: . 5.5 (-30.6%))	reduction of I-PPS score in early stages (Alken I and II) However no difference between plant extract and placebo group were registered. 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
Schiebel-Schlosser <i>et al.</i> 1998	Open study	Inclusion criteria: Stadium I and early II according to Alken <ul style="list-style-type: none"> prostatic volume 	1) Prosta Fink Forte capsule (500 mg of pumpkin seeds extract – 15-25:1 ethanol 92%	Age: 20 – 90 years 85% of	Side effects: gastrointestinal complains, hypotension,	1) I-PSS=10.94 (change: - (41.4%)) Statistically	Administration of pumpkin seeds extract for 3 month resulted in significant

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
		<ul style="list-style-type: none"> ●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>w/w)</p> <p>1 – 2 capsules/day (equivalent to 10g pumpkin seeds/day)</p> <p>3 months</p>	<p>patients in the age of 50 -80 years</p> <p>N=2245</p> <p>T-test</p>	<p>allergy</p> <p>No side effects were registered in 96% of patients</p> <p>χ^2 Test</p>	<p>significant Effects of treatment</p> <p>Quality of life (LQ index): 3.36 improvement (46.1%)</p>	<p>reduction of I-PPS score in early stages (Alken I and II) and improved quality of life</p>
Nitsch-Fitz <i>et al.</i> 1979	Open study,	<p>Inclusion criteria: Increased:</p> <ul style="list-style-type: none"> ●prostatic volume ●nocturia ●urinary flow rates ●residual urinary volume <p>Stage I according to Alken</p>	<p>1)Granulate of pumkin seeds <i>Cucurbita pepo</i> L. <i>convarietas citrullinina</i> I. GREB. <i>var. styriaca</i> (Kürbis-Granufink; in 100g = proteins 22 g, lipids 32 g,</p>	<p>N=43</p> <p>N= 39 at the end of the study (Exclusions: 3 patients Excluded after</p>	<p>Gastrointestinal complaints in 3 patients</p>	<p>Before the test 70% of patients complained of BPH symptoms, after 8 weeks therapy such complained only 20% of patients.</p>	<p>Administration of <i>Serenoa repens</i> and <i>Cucurbita pepo</i> seeds extract for 8 weeks months induced improvement of BPH symptoms.</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
			carboxyhydrates 38 g, phytosterols, vitamins (vit. E 20-30 mg%, aminoacids, metals-Fe, Mn, Zn, Cu, Se, cucurbitacin) 8 weeks	gastrointestinal complaints. Median age = 54 years No statistical data			
Hamvas <i>et al.</i> 1991	Open study	Inclusion criteria: 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 2x/day for 1 month/day, 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.

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
Auel 1962	Open study Presentation of 16 case studies in patients with BPH symptoms	Inclusion criteria: BPH symptoms: Increased ●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	
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 –(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 I female patients	

Table 10. 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	Open 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 3x/day 2) Placebo 3 months	N=26 1) 13 2) 13 1)52 – 80 mean 66.3 years; 2) 52-81 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	Open multicenter (6), randomized, double blind study, with placebo group	Inclusion criteria: Increased: ●prostatic volume ●voiding time (s) ●diurnal frequency ●nocturnal	1) Mixture of extract of <i>Serenoa repens</i> (80 mg) and <i>Cucurbita pepo</i> seeds (80 mg) (CURBICIN®) 2 tablets 3x/day	N=26 1) 13 2) 13	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.

		<p>frequency</p> <ul style="list-style-type: none"> •urinary flow rates (ml/s) •residual urinary volume<300 ml <p>Subiective variables:</p> <ul style="list-style-type: none"> • dysuria • patient's evaluation of therapy 	<p>2)Placebo</p> <p>3 months</p>	<p>1) 52 – 80 mean 66.3 years;</p> <p>2) 52-81 mean: 64.5 years</p> <p>Student T-test</p>			<p>Increase of urinary flow (3.0±4.0 ml/s, p<0.001;</p> <p>decrease of micturition time (2.4±3.6 s, p<0.01;</p> <p>decrease of residual volume (42.5±62.1 ml, p<0.01;</p> <p>decrease of diurnal frequency (1.0±1.5 , p<0.05;</p> <p>decrease of nocturnal frequency (0.6±0.7, p<0.01</p>
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Four studies admitted by the Hungarian National 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, nycturia 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 group of 20 boys of 2 – 7 years of age (70% with malnutrition) mixture of fresh, peeled and roasted pumpkin seeds were administered for 4 days to provide 60 mg/kg phosphorus/day. Pumpkin seeds lowered calcium-oxalate crystal occurrence and calcium levels in urine and increased phosphorus and potassium levels. 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 of Suphiphat *et al.* (1993), 10 adolescents (13 – 16 years of age) with urinary bladder stones disease were treated with pumpkin seeds snacks for two days at the level of 1.2 g of phosphorus/day. Pumpkin seeds induced inhibition of crystal formation of calcium-oxalate stones and raised pyrophosphate and magnesium excretion in urine. Elevation both pyrophosphate and magnesium would inhibit calcium oxalate precipitation in urinary tract.

Anthelmintic studies

To the 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 patients 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 open clinical study, including 150 patients with diagnosis of parasite infections 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 parasympathomimetic activity of arecoline present in areca nuts.

In other observational study, Feng (1956) found reliable therapeutic results in 96 patients infected with *T. 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: 60-125g 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 meeting WHO-BPH criteria, except one by Bach (2000). After 12 months of treatment, a significant change of the International Prostate Symptom Score compared to placebo was observed. The changes of Qmax, quality of life, prostate volume and postvoid residual volume were not different from placebo group.

Accordingly, the medicinal use of pumpkin seeds has to be regarded as traditional.

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

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

Allergy

Allergy to *Cucurbita pepo* was detected in four patients complaining of pruritus, nausea and diarrhea 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 2.5 year 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 min 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

Yu and Jansson (2001) described two cases of coagulation disorders with increased INR values that were associated with the herbal drug Curbicin (active constituents: extracts of *Serenoa repens*, *Cucurbita pepo* and vitamin E). The INR normalized after discontinuation of Curbicin treatment. However, pure vitamin E administration is related to prolongation of coagulation time, therefore a casual relationship for pumpkin extract in this interaction could not be established.

Pregnancy and lactation

No experimental studies are available.

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

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.

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 Community. 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 well-established use in this indication. Therefore the medicinal use of pumpkin seed has to be regarded as traditional in the sense of Dir. 2004/24/EC.

Indications for treatment: Traditional herbal medicinal product in micturition disorders. Supportive treatment in functional disorders of the bladder (irritable bladder with urgency, increased daytime and

night frequency and urge urinary incontinence).

In conclusion, pumpkin seeds can be used for the relief of lower urinary tract symptoms related to benign prostatic hyperplasia and/or related to an overactive bladder.

However pumpkin seeds can be only used in conditions which are controlled by a doctor with regular medical checks (Roehrborn 2008).

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

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

Annex

List of references