



DIPLOMARBEIT

Titel der Diplomarbeit

Evaluation of the traditional and well-established use of
Tormentillae rhizoma, Caryophylli flos and Caryophylli aetheroleum

angestrebter akademischer Grad

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1. INTRODUCTION

The European Medicines Agency (EMA) established a committee under the name Committee for Herbal Medicinal Products (HMPC) for the EU-wide harmonization of the state of knowledge on herbal medicinal products and medicinal plants. The HMPC has 32 members (one member per EU-member state and five co-opted members) and holds six meetings a year at the EMA in London.

One of the legal mandates of the HMPC is the establishment of community monographs on herbal substances and herbal preparations according to well-established use and traditional use.

Definition of Well-established use according to Dir. 2001/83 as amended:

- A defined herbal preparation is in broad medicinal use within the European Union since at least 10 years
- The clinical efficacy in the claimed indication is supported by at least one controlled clinical study of good methodological quality
- There is coherence of the scientific assessment
- The high degree of scientific interest in the use of the preparation is reflected in the published scientific literature

Definition of Traditional use according to Dir. 2001/83 as amended:

- The herbal preparation or a corresponding product is in medicinal use for at least 30 years, from this at least 15 years within the European Union
- Exclusively in indications which do not need medical diagnosis or medical supervision
- The efficacy must be plausible
- Route of administration: exclusively for oral use, cutaneous use or inhalation

The use of medicinal plants and products thereof is highly different throughout the European Union. Despite existing monograph collections such as ESCOP monographs, pharmacopoeia monographs or the Commission E monographs, national barriers remain which is in contrast to the main principles of the harmonised markets within the European Union. These barriers should be eliminated by the creation of HMPC monographs.

The creation of Community herbal monographs has the goal of establishing a common basis within the EU for the authorisation and registration of herbal medicinal products. A uniform scientific basis is to be established.

Monographs are to be considered by Member States in the assessment of an application for authorisation or registration and therefore also represent a service for companies or applicants. Provided that a herbal medicinal product complies with the monograph the applicant is not obliged to provide the complete literature on safety, efficacy or traditional use. The application dossier is more or less restricted to the quality part.

Unfortunately community monographs are not legally binding at the moment. However, the international pressure on member states not accepting monograph conform applications is increasing.

Austria is among the leading member states in the level of acceptance of monographs and also intensively involved in the development of such monographs. Austria is among other herbal substances responsible for the monographs on *Potentilla erecta* L. and *Syzygium aromaticum* L.

This diploma thesis should focus on the search and discussion of scientific literature on *Potentilla erecta* L. and *Syzygium aromaticum* L. It presents a preliminary draft paper on the well-

established use (based on article 10A of directive 2004/24/EC) and the traditional use (based on article 16D (1) of directive 2004/24/EC) for the Committee for Herbal Medicinal Products of the European Medicines Agency (EMA).

2. METHODOLOGY

2.1. LITERATURE SEARCH:

Period: August 2009 to January 2010

Electronic data search:

Databases:

- Pubmed (<http://www.ncbi.nlm.nih.gov/sites/entrez>)
- Toxnet (<http://www.toxnet.nlm.nih.gov/>)
- Medline

Keywords:

Potentilla erecta: *Potentilla erecta*, *Potentilla tormentilla*, tormentil, Blutwurz

Syzygium aromaticum: *Syzygium aromaticum*, Gewürznelke, Caryophylli flos, Eugenol

Relevant articles were identified and obtained in full text.

Libraries:

University Vienna, center of pharmacy; Medical University Vienna, central library

The center of pharmacy of the University of Vienna, the Medical University of Vienna and the central library are adequate sources of older handbooks of pharmacy, old pharmacopoeias and old handbooks of phytotherapy. These sources are essential for the documentation of the traditional use.

2.2. PROCEDURE FOR THE DEVELOPMENT OF COMMUNITY MONOGRAPHS

The procedure for the establishment of community monographs is defined by standard operations procedures (SOPs) from the EMA. The main steps are:

- Appointment of a rapporteur and a peer-reviewer
- Literature search by the rapporteur
- Collection of data concerning authorised medicinal products in the EU member states regarding date of authorisation, composition, indication, and posology
- Assessment of the scientific literature and the period of medicinal use
- Development of a draft assessment report, a draft monograph and a draft list of references
- Discussion(s) in the HMPC working party on monographs and lists (MLWP) and revision of the documents
- Adoption of the documents by the HMPC for release for public consultation
- Consultation phase of three months
- Interested parties are invited to comment on the draft monograph
- Rapporteur to collect and assess the comments, documents are modified accordingly; rejection of comments has to be justified by the rapporteur
- Peer review of the documents
- Adoption of the final documents by the HMPC
- Publication on the website of the EMA

At the moment of the compilation of this diploma thesis the draft documents on *Tormentillae rhizoma* are published for consultation. The development of the monograph and assessment report on *Caryophylli flos* and *Caryophylli aetheroleum* are in the stage before the first discussion in the MLWP.

The HMPC provides guidance documents on the evaluation of literature and the preparation of the documents. The style and the structure of the documents are defined in templates provided by the HMPC. Guidelines and templates are available at the website of the EMA (<http://www.ema.europa.eu/htms/human/hmpc/index.htm>).

3. RESULTS

3.1. POTENTILLA ERECTA (L.) RAEUSCH., RHIZOMA

London, January 2010
Doc. Ref.: EMA/HMPC/5511/2010

COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

ASSESSMENT REPORT ON POTENTILLA ERECTA (L.) Raeusch., RHIZOMA¹

¹ Botanical name of the plant according to the binomial system (genus, species, variety and author), [comma] the plant part in Latin.

REGULATORY STATUS OVERVIEW²

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'

Member State	Regulatory Status				Comments ³
Austria	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Belgium	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input checked="" type="checkbox"/> Other Specify:	Food supplements
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Combinations only
Denmark	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Food supplements
Estonia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No product
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
France	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Germany	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Greece	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No product
Italy	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No product
Latvia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input checked="" type="checkbox"/> Other Specify:	Combination only
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Lithuania	<input type="checkbox"/> MA	<input checked="" 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:	no response
Malta	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Norway	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Poland	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
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:	no response
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:	no response
Spain	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input checked="" type="checkbox"/> Other Specify:	Tincture (1:10)
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:	No product

² This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

³ Not mandatory field

ASSESSMENT REPORT

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

(WELL-ESTABLISHED USE)

BASED ON ARTICLE 16D(1), ARTICLE 16F AND ARTICLE 16H OF DIRECTIVE 2001/83/EC AS AMENDED

(TRADITIONAL USE)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Potentilla erecta, rhizoma Syn: Tormentillae rhizome
Herbal preparation(s)	A) Comminuted herbal substance B) Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 70% (v/v) C) Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 45% v/v D) Liquid extract (DER 1:1, extraction solvent ethanol 25% v/v)
Pharmaceutical forms ⁴	Comminuted herbal substance as herbal tea for oral and oromucosal use. Herbal preparations B, C and D in liquid dosage forms for oral use. Herbal preparation B in liquid dosage forms for oromucosal use.
Rapporteur ⁵	Austria
Assessor(s) ⁶	Reinhard Länger, Birgit Hochenegg

⁴ Pharmaceutical form only according to EDQM standard term

⁵ Name of HMPC/MLWP member (not Member State)

⁶ To be deleted at publication stage, unless Rapporteur requests to keep the name(s)

3.1.1. Introduction

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

Herbal substance(s) 7:

Tormentillae rhizoma consists according to the European Pharmacopoeia of the dried rhizome, freed from the roots of *Potentilla erecta* (L.) Raeusch. It contains not less than 7 per cent of tannins, expressed as pyrogallol ($C_6H_6O_3$, Mr126.1) with reference to the dried herbal substance.

Constituents (according to Hänsel & Sticher 2007, Blaschek et al 2008, Wichtl 2009):

Tannins: 15-22% total tannins; about 70% of them are condensed tannins like catechins (–)- gallo- or (–)-epigallocatechingallat, the dimeric catechin derivatives [6,6']-all-trans-bi-(+)-catechin, [4,8]-all-trans-bi-(+)-catechin (= procyanidin B3), [4,6]-all-trans-bi-(+)-catechin (= procyanidin B6) and [4,8]-2,3-trans-3,4-cis-bi-(+)-catechin and 3,5% hydrolysed like gallo- and ellagitannine gallic acid, ellagic acid and the main component of the hydrolysed tannins is the dimeric agrimoniin, which is contained to about 1% in the drug. Further HHDP = hexahydroxy diphenic acid), pedunculagin, laevigatin B und laevigatin F were isolated.

Flavonoids: kaempferol, cyanidinglucoside and leucoanthocyanidin and the tannin monomers catechin, epicatechin, gallocatechin and epigallocatechin

Phenol carboxylic acids: p-coumaric acid, 3,4-dihydroxybenzoic acid, gallic acid, sinapic acid and caffeic acid

Triterpene saponins: quinovic acid, tormentillic acid and tormentosid (glycoside of tormentillic acid)

Fatty acids: in extracts prepared with supercritical CO_2 the following constituents are found: lauric acid, linoleic acid, linolenic acid, palmitic acid, palmitoleic acid, pentadecanoic acid, stearic acid and oleic acid.

Herbal preparation(s):

The following herbal preparations are in medicinal use in the community for more than 30 years:

	Herbal preparation	References
A	Comminuted herbal substance. for preparation of infusions for preparation of decoctions (authorized products in Poland, on the market for more than 30 years)	Madaus 1938
B	Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 70% (v/v)	Monograph in the Czech pharmacopoeia since 1970, monograph in the Austrian pharmacopoeia at least since 1960, replaced by the monograph in Pharm. Eur. 01/2008: 1895.
C	Tincture (ratio drug : extraction solvent 1:5), extraction	British Herbal Pharmacopoeia,

6 According to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMA/HMPC/182320/2005 Rev.2) and the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use (EMA/HMPC/182352/2005 Rev.2)

	solvent ethanol 45% v/v	1974 Explanation missing
D	Liquid extract (DER 1:1, extraction solvent ethanol 25% v/v)	British Herbal Pharmacopoeia, 1974 Explanation missing Waiting for Linda earlier editions
E	Dry extract (DER 3.5-4.5:1, extraction solvent ethanol 60% v/v)	Authorised product in Germany, on the market at least since 1976

Herbal preparations not considered in the monograph:

Powdered herbal substance:

Traditionally suspended in red wine for the treatment of acute unspecific diarrhoea (Wichtl 2009). Although the combination with tannins from red wine seems to be plausible is this special pharmaceutical form not suitable for THMPs. Weiß (1985) proposes a pinch of the powdered herbal substance several times daily. This posology seems to be too imprecise.

Tincture (ratio drug : extraction solvent 1:10), extraction solvent ethanol 70% (v/v)

This herbal preparation is mentioned only in recent editions of handbooks on phytotherapy (e.g., Jänicke et al 2003, Kraft 2000). It seems that the authors refer in the proposed posology to the tincture (1:5).

For the tincture (1:10) mentioned in the market overview of Spain no further information on extraction solvent and posology is available.

Combinations of herbal substance(s) and/or herbal preparation(s)⁸

Tormentillae rhizoma and its preparations are used with many other herbal substances or herbal preparations. This monograph refers only to Tormentillae rhizoma.

Vitamin(s)⁹

Not applicable

Mineral(s)¹⁰

Not applicable

Information about products on the market in the Member States

The following information has been provided regarding monographs in national pharmacopoeias or products on the market with relevance for this monograph:

CZ:

Tormentillae radix has been a subject of Czechoslovak/Czech Pharmacopoeia since 1970; recommended dosage in the last version of the Czech Pharmacopoeia: single dose for oral use 1.5 g, daily dose for oral use 4.0 – 6.0 g.

Tormentillae tinctura (ratio drug : extraction solvent 1:5), extraction solvent ethanol 70% (v/v), has been a subject of Czechoslovak/Czech Pharmacopoeia since 1970; recommended dosage in the last version of the Czech Pharmacopoeia: single dose for local use 0.5 -1.0 g

⁸ According to the 'Guideline on the clinical assessment of fixed combinations of herbal substances/herbal preparations' (EMA/HMPC/166326/2005)

⁹ Only applicable to traditional use

¹⁰

DK:

The Danish Food Agency has accepted 200 mg *Potentilla erecta*, radix in a food supplement. This is not an upper limit but a specific assessment in a specific case.

LI:

The comminuted herbal substance is on the market since more than 30 years. The products are for the preparation of infusions for use in the proposed indications.

PL:

The comminuted herbal substance is on the market since more than 30 years. The products are for the preparation of decoctions for use in the proposed indications.

Search and assessment methodology

- Search terms: *Potentilla erecta*, *Potentilla tormentilla*, tormentil, Blutwurz
- Databases: Pubmed, Medline and Toxnet.
- Libraries: University Vienna, center of pharmacy; Medical University Vienna, central library.

Historical data on medicinal use

Information on period of medicinal use in the Community

The medicinal use of *Tormentillae rhizoma* can be traced in literature back to the 15th century (according to Madaus 1938), it is also mentioned by Lonicerus and Matthiolus in the 17th century (cited in Benedum et al 2006).

In fact *Tormentillae rhizoma* has been in therapeutic use for many decades, a continuous use during the last 30 years is documented in the literature.

Therefore for *Tormentillae rhizoma* a period of at least 30 years in medicinal use as requested by Directive 2004/24 EC for qualification as a traditional herbal medicinal product is easily fulfilled.

The type of tradition: European.

Information on traditional/current indications and specified substances/preparations

Tormentillae rhizoma is traditionally used for acute, unspecified diarrhoea, externally for haemostasis, mild inflammation of the oral and pharyngeal mucosa, prosthetic pressure points, frostbites, burnings, haemorrhoids and poorly healing wounds (Madaus 1983, List & Hörhammer 1977, British Herbal Pharmacopoeia 1983, Weiß 1985, Blaschek et al 2008, Fintelmann & Weiss 2002, Augustin & Hoch 2004).

Proposed indications for traditional use:

Indication 1:

Traditional herbal medicinal product for short-term use in cases of acute, unspecified diarrhoea.

Indication 2:

Traditional herbal medicinal product for the symptomatic treatment of inflammations in the mouth or the throat.

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

The high content of tannins makes the medicinal use in the proposed indication plausible.

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

Posology

Indication 1: Diarrhoea

	Herbal preparation	Posology
A	Comminuted herbal substance. for preparation of infusions: for preparation of decoctions:	Corresponding 4-6 g herbal substance daily (authorised products in Lithuania since more than 30 years) 2-4 g thrice daily (British Herbal Pharmacopoeia 1974) Corresponding 4-6 g herbal substance daily (German Commission E cited in Blumenthal 1998, Wichtl 2009) 2-3 g in 150 – 200 ml of water: drink 2 x daily (authorised products in Poland since more than 30 years)
B	Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 70% (v/v)	30 – 50 drops in water, several times daily (Weiß 1985)
C	Tincture: DER 1:5, extraction solvent alcohol 45% v/v	2-4 ml thrice daily (British Herbal Pharmacopoeia 1974)
D	Liquid extract: DER 1:1, extraction solvent alcohol 25% v/v	2-4 ml thrice daily (British Herbal Pharmacopoeia 1974)
E	Dry extract (DER 3.5-4.5:1, extraction solvent ethanol 60% v/v)	3 times daily 2 capsules, each containing 200 mg dry extract

Indication 2: inflammations in the mouth or the throat

	Herbal preparation	Posology
A	Comminuted herbal substance. for preparation of infusions for preparation of decoctions	Corresponding 4-6 g herbal substance daily (authorised products in Lithuania since more than 30 years) 2-3 spoons (= 8-12 g) of the rhizome per litre water. Rinse the mouth several times daily (Fintelmann & Weiss 2002). Corresponding 4-6 g herbal substance daily (German Commission E cited in Blumenthal 1998, Wichtl 2009) 6 g in 200 ml water: for washing oral cavity (authorised products in Poland since more than 30 years)

		30 years)
B	Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 70% (v/v)	1 teaspoon per glass of water, rinse the mouth (Fintelmann & Weiss 2002). 10-20 drops to one glass of water daily (Blumenthal et al 1998)
C	Tincture: DER 1 :5, extraction solvent alcohol 45% v/v	no posology available
D	Liquid extract : DER 1 :1, extraction solvent alcohol 25% v/v	no posology available
E	Dry extract (DER 3.5-4.5:1, extraction solvent ethanol 60% v/v)	Not authorised for this indication

Use in children:

There are no data from clinical trials or observational trials available. Therefore the use should be restricted to adults.

The use of the dry extract was allowed in Germany for adolescents in case of unspecific acute diarrhoea.

Duration of use

Indication 1:

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

Indication 2:

If the symptoms persist longer than 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Method of administration

Indication 1:

Oral use

Indication 2:

Oromucosal use

3.1.2. Non-Clinical Data

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

Data obtained with traditional herbal preparations relevant for the proposed indications:

Antiviral effect:

A decoction (DER 1:10) revealed antiviral activity in a plaque inhibition test against Herpes virus HVP 75 (type 2) and vaccine virus (May & Willuhn 1978).

Further data:

Astringent effect:

Tannins as polyphenols exhibit the potential for so called multidentate interactions with other molecules, predominately with proteins. The binding on proteins may be irreversible (covalent binding) or reversible

(hydrogen bonds). The astringency results from this affinity for proteins. Externally, they waterproof the external layers of the skin and mucosas, thus protecting the underlying layers; they also have a vasoconstrictor effect on small superficial vessels (Hänsel & Sticher 2007). Thus, the absorption of fluids from the intestinal lumen and the influx into this is reduced, the precipitate of the protein-tannin complex serves as a protective layer. The absorption of toxins is impeded; the effects of local irritants are reduced (Dingermann & Loew 2003). For most of these presumptions no experimental data are available.

Antimicrobial effects:

Tannins exhibit antimicrobial effects independent of their plant source (Hänsel & Sticher 2007). A tannin complex isolated from Tormentillae rhizome, which contained tannins, sugar and triterpenes, prevented the growth of the following bacteria fully in the following concentrations : 2,5 mg/ml : Pasteurella pseudotuberculosis, Shigella boydii, Shigella flexneri, Shigella sonnei; 5mg/ml: Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus; 10mg/ml: Escherichia coli 055B5, E. coli 0111B4, streptococcus faecalis (Pourrat et al 1963 cited in Blaschek et al 2008). The mentioned concentrations may be reached after consumption of a herbal tea of tormentil.

Antiinflammatory effect:

Antiinflammatory properties are documented for a wide variety of tannins (Scholz 1994). Agrimoniin, a major tannin of tormentil, was identified as a potent direct inhibitor of human neutrophil elastase with an IC_{50} of 0.9 μ M (Hrenn et al 2006).

Immunostimulatory effect:

A weak immunostimulating effect was detected with a crude extract prepared with water containing acetone. To mice 25 mg crude drug extract per kg bodyweight were applied intraperitoneally. After an hour they were given oxazolone to sensitization on the shaved belly. After 7 days oxazolone was applied on one ear, which led to an average increase of the ear thick by 24 %. At an increase by 50 %, a substance is considered active by the authors. No further details are published regarding the number of mice or statistical parameters (Lund & Rimpler 1985).

Drozd & Anuszevska (2005) demonstrated that the combined addition to a culture of decoctions from tannin pharmacopoeial raw materials, containing ellagic acid (Cortex Quercus, Folium Uvae ursi and Rhizoma Tormentillae, decoction in usual concentration = app. 2 g tormentil per 150 ml water) and the antibiotics: cefuroxime, cefoperazone and doxycycline influences the enhancement of survival of mouse thymocytes in cultures with supplementation of hydrocortisone. A cytotoxic test was used for the evaluation. The presented results confirmed that each of the applied aqueous extracts shows a better survival of mouse thymocytes after combined addition of an antibiotic to the culture. The extract from Cortex Quercus showed the highest activation when added to the culture with cefuroxime, whereas cefoperazone best stimulation demonstrated when combined with the extract from Rhizoma Tormentillae, and doxycycline with the extract from Folium Uvae ursi. The authors conclude that the combination of antibiotics and tannin raw materials could be advantageous for the immunological system of patients.

Assessor's comment: the interpretation of the authors seems to be too optimistic. The conclusions made are far away from practical consequences.

Interferon inducing effect:

A weak effect was observed in a study on interferon induction. Mice received 100 mg crude drug extract per kg bodyweight intraperitoneally. After 17 hours 0,05 ml of a dilution of Vaccinia-virus (IHD strain) was applied intravenously. This concentration produced untreated 12 to 25 separate lesions on the tail of each animal. Inhibition of these lesions on the eighth day by more than 50 % is interpreted as a possible interferon inducing effect. The observed inhibition was approximately 28 %. No further details are published regarding the number of mice or statistical parameters (Lund & Rimpler 1985).

Molluscicide effect :

Aqueous and methanol extracts (5 g herbal substance in 100 ml) in concentrations of 400 ppm or 100 ppm are still active against the freshwater snail *Biomphalaria glabrata*, which is the intermediate host of schistosomiasis. The molluscicide principle of the drug are the tannins (Schaufelberger & Hostettmann 1983).

Hypoglycemic effect :

Tormentic acid (isolated from the plant *Poterium ancistroides*, but also a constituent of tormentil) lowered in a concentration of 30 mg/kg BW the fasting plasma glucose level with a corresponding increase in circulating insulin levels in rats. It also improved the glucose tolerance test by increasing insulin secretory response to glucose. Tormentic acid did not alter the insulin and glucose levels in streptozotocin-induced diabetic rats (Ivorra et al 1988). Since no data on the concentration of tormentic acid in tormentil are available, the relevance of this publication for the use of tormentil cannot be assessed.

Antioxidant activity:

Dimers and trimers of procyanidins of tormentil displayed the highest anti-radical activity towards lipoperoxidation compared to other fractions of a water soluble tormentil extract. The IC_{50} of a total extract was determined with 0.024 mg/ml. Pentamers and hexamers possessed the most marked anti-elastase properties. The IC_{50} of a total extract was determined with 0.044 mg/ml. These effects are interpreted by the authors as a possible prevention of the aging effects of oxidative membrane impairment. No standard antioxidants were included for comparison of the results (Vennat et al 1994, Bos et al 1996).

Chandak et al (2009) investigated pure gallotannin in rats with Streptozotocin (STZ)-induced diabetic nephropathy. Poly(ADP-ribose) polymerase (PARP) is known to be activated under conditions of oxidative stress and/or radiation exposure. Inhibition of PARP by specific inhibitors is known to prevent the development of STZ induced diabetic nephropathy by reduction in oxidative stress induced apoptosis. Gallotannin (20 mg/kg/day, i.p.) treatment for 4 weeks led to a significant reduction in the levels of plasma creatinine which is a well known marker for diabetic nephropathy. Treatment with gallotannin resulted in protection up to a certain level of glomerular damage, suggesting compensatory glomerular hypertrophy. As a PARP inhibitor gallotannin treatment also showed protection in PARP cleavage which is a hallmark for apoptotic cell death signifying the protective role of gallotannin in cell death signalling.

Tormentil (no details on the herbal preparation and concentration) showed antioxidant activity in a cell-free oxidant-generating system as well as in sigmoid or rectal mucosal biopsies obtained from patients with active ulcerative colitis (Langmead et al 2002).

Effect on histamine release:

The effects of tannins and related polyphenols on potassium superoxide- and compound 48/80-induced histamine release from rat peritoneal mast cells were examined. Pre-treatment with hydrolysable tannins (1-100 μ M) significantly inhibited potassium superoxide-induced histamine release. For example agrimoniin inhibited the potassium superoxide- induced histamine release with an IC_{50} of 0.68 μ M, the compound 48/80-induced histamine release with an IC_{50} of 0.49 μ M. The inhibitory effect on histamine release caused by different stimulants suggested that ellagitannins act as cell membrane stabilizers as well as radical scavengers (Kano et al 2000).

Antitumor effects:

Agrimoniin (concentration app. 1% in the herbal substance) was identified as a potential antitumor agent by Miyamoto et al (1987). Agrimoniin rejected almost completely the growth of ascites type and solid type tumours in mice in a dose of 10 mg/kg when applied intraperitoneally. Murayama et al (1992) found that agrimoniin induces the interleukin-1 secretion dose-dependently which was interpreted as possible mechanism for the antitumor activity.

Miyamoto et al (1988) found that agrimoniin in a dose of 10 mg/kg enhanced in vitro the cytotoxic potential of several effector peritoneal exsudate cells with different induction kinetics. The earlier response is an enhanced NK cell activity of nonadherent peritoneal exsudate cells, later responses were enhanced cytotoxic activity of adherent cells and an antibody-dependent macrophage-mediated cytotoxic activity.

Assessor's comment: Considering the low amount of agrimoniin in the herbal substance (about 1%) these findings seem to be of minor relevance for the oral and oromucosal use of tormentil.

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

No specific data are available on *Tormentillae rhizoma*.

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

Acute Toxicity:

Dry extract (extraction solvent acetone/water 75:25): in dosages of 300 mg/kg p.o. and 200 mg/kg i.p. no signs of toxicity in mice (Lund & Rimpler 1985).

A dry extract prepared by maceration with water was applied to rats and mice by the intragastric route in dosages of 2.5 g/kg and 6.8 g/kg respectively. Further, a single dose was applied intraperitoneally in dosages of 3.8 g/kg in mice and 14.5 g/kg in rats. No signs of toxicity could be observed, the macroscopical and microscopical investigation of the internal organs revealed no pathological changes. The tested doses correspond to several hundreds of grams of extract for a person with 75 kg (Shushunov et al 2009).

No tests on genotoxicity, carcinogenicity and reproductive toxicity have been performed.

Tannins may have carcinogenic potential; this is for example documented for oak. It is not clear whether these findings are relevant for tormentil too.

Overall conclusions on non-clinical data

The astringent effect of the tannins makes the use of tormentil plausible in the proposed indications. Although the data on toxicology are limited there are no safety concerns for the use as traditional herbal medicinal product.

3.1.3. Clinical Data

Clinical Pharmacology

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

There is no data available.

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

In the study of Huber et al (2007, see II.4.2.1) neither undegraded nor metabolized tannins could be detected by LC-MS in 15 patient sera even after application of 3000 mg/d of an ethanolic dry extract. No data are available regarding further constituents of tormentil.

Clinical Efficacy

Dose response studies

In an open-label study Huber et al (2007) investigated the safety, pharmacology and clinical effects of different doses of a commercial ethanolic dry extract (tannin content 15-22%, no further data available). 15 patients with active ulcerous colitis finished the study. During treatment with Tormentil extracts, the CAI (Colitis activity index) was reduced from a mean of 8.3 to 3.9 when participants took 2400 mg/d, which was statistically significant. In addition, stool frequency, bloody stool and C-reactive protein decreased. Although the posology was very high the reported adverse effects were mild (heartburn, stomach discomfort, nausea).

Clinical studies (case studies and clinical trials)

There is no data available.

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

40 children in the age from 3 months to 7 years suffering from a rotavirus diarrhoea were included in a clinical trial by Subbotina et al (2003). The children in the active group received 3 drops tormentil extract per year of life three times daily until discontinuation of the diarrhoea or at maximum for 5 days. The study medication was a tincture (1:10, extraction solvent ethanol 40%). The placebo was an alcohol extract of Indian teas which were identical in appearance and taste with tormentil tincture. The duration of the diarrhoea was in the treatment group 3 days, in the placebo group 5 days. No side effects were reported.

Assessor's comment:

The data support the traditional use of tormentil for the treatment of diarrhoea.

Overall conclusions on clinical pharmacology and efficacy

The published clinical trials give only preliminary data. They support the traditional use of tormentil. However, the level of evidence does not support well-established use.

3.1.4. Clinical Safety/Pharmacovigilance

Overview of toxicological/safety data from clinical trials in humans

There is no data available.

Patient exposure

None reported.

Adverse events and serious adverse events and deaths

The only source of documented adverse effects is the study by Huber et al (2007). All side effects were mild, although 62 % experienced mild gastrointestinal symptoms. One of the 15 patients developed worsening of colitis and was hospitalized.

Laboratory findings

There is no specific data available.

Safety in special populations and situations

There is no specific data available.

Overall conclusions on clinical safety

The medicinal use of *Tormentillae rhizoma* can be regarded as safe.

3.1.5. Overall Conclusions

Risk – benefit assessment:

Since no specific risks are known regarding the oral and oromucosal use of herbal preparations of tormentil, there are no limitations from the herbal preparation when used in adults.

Persistent diarrhoea may cause dehydration and loss of electrolytes. Rehydration and substitution of electrolytes are the primary therapeutic goals. Acute, unspecific diarrhoeas are in most cases self-limiting diseases, a supportive treatment with astringents like tormentil may help to reduce the duration and the severity of the complaints. However, if the symptoms persist for more than 2 days the diarrhoea should be treated and monitored by a doctor.

Only limited data are available regarding the use of tormentil in children and adolescents in the case of diarrhoea. Astringents like purified tannins are recommended in standard literature for children because of their safety compared to therapeutic alternatives (Mutschler et al 2008). However, acute diarrhoea may be life-threatening especially for young children when not properly treated. Therefore the use of tormentil in the case of diarrhoea in children is not suitable for a traditional herbal medicinal product.

The use as a gargle in the case of inflammations in the mouth does not show any limitations. However, there are no data regarding a posology in children and adolescents available. Therefore the use should be limited to adults.

No data are available on the safe use during pregnancy and lactation. Therefore the use of tormentil is not recommended during pregnancy and lactation.

ANNEXES:

3.1.6. Community Herbal Monograph on *Potentilla erecta*, rhizoma¹¹

London, 13 January 2010
Doc. Ref.: EMA/HMPC/5513/2010
MLWP changes

COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

COMMUNITY HERBAL MONOGRAPH ON *POTENTILLA ERECTA* (L.) RAEUSCH, RHIZOMA

DISCUSSION IN WORKING PARTY ON COMMUNITY MONOGRAPHS AND COMMUNITY LIST (MLWP)	<i>January 2010</i>
ADOPTION BY HMPC FOR RELEASE FOR CONSULTATION	
END OF CONSULTATION (DEADLINE FOR COMMENTS)	
REDISCUSSION IN WORKING PARTY ON COMMUNITY MONOGRAPHS AND COMMUNITY LIST (MLWP)	
ADOPTION BY HMPC	

Comments should be provided using this [template](#) to hmpc.secretariat@emea.europa.eu
Fax: +44 20 75 23 70 51

KEYWORDS	Herbal medicinal products; HMPC; Community herbal monographs; traditional use; <i>Potentilla erecta</i> (L.) Raeusch., rhizoma; tormentil
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BG (bългарски): CS (čeština): DA (dansk): DE (Deutsch): Tormentillwurzelstock, Blutwurz EL (ελληνικά): EN (English): ES (español): ET (eesti keel): FI (suomi): FR (français): HU (magyar): IT (italiano):	LT (lietuvių kalba): LV (latviešu valoda): MT (malti): NL (nederlands): PL (polski): PT (português): RO (română): SK (slovenčina): SL (slovenščina): SV (svenska): <i>IS (íslenska):</i> <i>NO (norsk):</i>
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¹¹ Prepared according to the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use' (EMA/HMPC/182352/2005 Rev.2)

COMMUNITY HERBAL MONOGRAPH ON POTENTILLA ERECTA (L.) RAEUSCH, RHIZOMA

NAME OF THE MEDICINAL PRODUCT

To be specified for the individual finished product.

QUALITATIVE AND QUANTITATIVE COMPOSITION¹²¹³

<u>Well-established use</u>	<u>Traditional use</u>
With regard to the marketing authorisation application of Article 10(a) of Directive 2001/83/EC as amended	With regard to the registration application of Article 16d(1) of Directive 2001/83/EC as amended Potentilla erecta (L.) Raeusch., rhizoma (tormentil) Herbal substance not applicable Herbal preparations A) Comminuted herbal substance B) Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 70% (v/v) ¹⁴ C) Tincture (ratio drug : extraction solvent 1:5), extraction solvent ethanol 45% v/v D) Liquid extract (DER 1:1, extraction solvent ethanol 25% v/v) E) Dry extract (DER 3.5-4.51, extraction solvent ethanol 60% v/v)

PHARMACEUTICAL FORM

<u>Well-established use</u>	<u>Traditional use</u>
	Comminuted herbal substance as herbal tea for oral use. Comminuted herbal substance for infusion or decoction preparation for oromucosal use. Herbal preparations B, C, D and E in liquid dosage forms for oral use. Herbal preparation B in liquid dosage forms for oromucosal use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term.

¹² The material complies with the Eur. Ph. monograph (ref.: 01/2008: 1478).

¹³ The declaration of the active substance(s) for an individual finished product should be in accordance with relevant herbal quality guidance.

¹⁴ The tincture complies with the Eur. Ph. monograph (ref.: 01/2008: 1895).

CLINICAL PARTICULARS

Therapeutic indications

<u>Well-established use</u>	<u>Traditional use</u> <u>Indication 1:</u> Traditional herbal medicinal product for symptomatic treatment of mild diarrhoea. <u>Indication 2:</u> Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the oral mucosa. The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.
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Posology and method of administration

<u>Well-established use</u>	<u>Traditional use</u> Posology <u>Indication 1 (oral use):</u> <i>Adults, elderly:</i> A) Comminuted herbal substance: As infusion: single dose 1.4-4 g, several times daily up to a maximum daily dose of 12 g comminuted herbal substance. As decoction: single dose 1.4-3 g, several times daily up to a maximum daily dose of 6 g comminuted herbal substance. B) Single dose: 1-2 ml in water; 3 times daily. C) Single dose 2-4 ml, 3 times daily. D) Single dose 2-4 ml, 3 times daily. E) Single dose 400 mg, 3 times daily. The use in children and adolescents under 18 years of age is not recommended (see section 4.4 'Special warnings and precautions for use'). <u>Indication 2 (oromucosal use):</u> <i>Adults, elderly:</i> A) Comminuted herbal substance: As infusion: 1.3 – 2 g per 100 ml of water.
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	<p>As decoction: 0.8 – 3 g per 100 ml of water.</p> <p>Rinse the mouth several times daily.</p> <p>B) Single dose: 1-5 ml per 150 ml of water.</p> <p>Rinse the mouth several times daily.</p> <p>The use in children and adolescents under 18 years of age is not recommended (see section 4.4 ‘Special warnings and precautions for use’).</p> <p>Duration of use</p> <p><u>Indication 1:</u> If the symptoms persist longer than 3 days during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.</p> <p><u>Indication 2:</u> If the symptoms persist longer than 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.</p> <p>Method of administration</p> <p><u>Indication 1:</u> Oral use.</p> <p><u>Indication 2:</u> Oromucosal use.</p>
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Contraindications

	<p><u>Traditional use</u> Hypersensitivity to the active substance</p>
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Special warnings and precautions for use

<u>Well-established use</u>	<p><u>Traditional use</u> <u>Indication 1:</u> If recurrent diarrhoea or bloody stools occur a doctor or a qualified health care practitioner should be consulted.</p> <p><u>Indications 1 and 2:</u> The use in children and adolescents under 18</p>
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	<p>years of age has not been established due to lack of adequate data.</p> <p>If the symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.</p> <p>For tinctures containing ethanol, the appropriate labelling for ethanol, taken from the ‘Guideline on excipients in the label and package leaflet of medicinal products for human use’, must be included.</p>
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Interactions with other medicinal products and other forms of interaction

<u>Well-established use</u>	<p><u>Traditional use</u></p> <p><u>Indication 1:</u> Internal absorption of concomitantly administered medicine may be delayed. For this reason the product should be taken 1 hour or more before or after intake of other medicinal products.</p> <p><u>Indication 2:</u> None reported.</p>
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Pregnancy and lactation

	<p><u>Traditional use</u></p> <p>Safety during pregnancy and lactation has not been established.</p> <p>In the absence of sufficient data, the use during pregnancy and lactation is not recommended.</p>
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Effects on ability to drive and use machines

<u>Well-established use</u>	<p><u>Traditional use</u></p> <p>No studies on the effect on the ability to drive and use machines have been performed.</p>
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Undesirable effects

<u>Well-established use</u>	<p><u>Traditional use</u></p> <p><u>Indication 1:</u> Mild stomach complains with the symptoms</p>
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	<p>nausea and vomiting may occur in sensitive patients. The frequency is not known.</p> <p>If other adverse reactions not mentioned above occur, a doctor or a qualified health care practitioner should be consulted.</p> <p><u>Indication 2:</u> None known.</p> <p>If adverse reactions occur, a doctor or a qualified health care practitioner should be consulted.</p>
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Overdose

<u>Well-established use</u>	<u>Traditional use</u> No case of overdose has been reported.
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PHARMACOLOGICAL PROPERTIES

Pharmacodynamic properties

Well-established use	<u>Traditional use</u> Not required as per Article 16c(1)(a)(iii) of Directive 2001/83/EC as amended.
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Pharmacokinetic properties

<u>Well-established use</u>	<u>Traditional use</u> Not required as per Article 16c(1)(a)(iii) of Directive 2001/83/EC as amended.
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Preclinical safety data

<u>Well-established use</u>	<u>Traditional use</u> Not required as per Article 16c(1)(a)(iii) of Directive 2001/83/EC as amended, unless necessary for the safe use of the product. Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed.
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PHARMACEUTICAL PARTICULARS

<u>Well-established use</u>	<u>Traditional use</u>

DATE OF COMPILATION/LAST REVISION

13.01.2010

3.2. SYZYGIUM AROMATICUM (L.) MERRILL. ET L.M. PERRY, FLOS, SYZYGIUM AROMATICUM (L.) MERRILL. ET L.M. PERRY, AETHEROLEUM

**COMMITTEE ON HERBAL MEDICINAL PRODUCTS
(HMPC)**

**ASSESSMENT REPORT ON
SYZYGIUM AROMATICUM (L.) MERRILL. ET L.M. PERRY, FLOS,
SYZYGIUM AROMATICUM AETHEROLEUM¹⁵**

¹⁵ Botanical name of the plant according to the binomial system (genus, species, variety and author), [comma] the plant part in Latin.

REGULATORY STATUS OVERVIEW¹⁶

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'

Member State	Regulatory Status				Comments ¹⁷
Austria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Combinations only
Belgium	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input checked="" type="checkbox"/> Other Specify:	Food supplements
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Denmark	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Estonia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
France	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Germany	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Greece	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Combinations only
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Italy	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Latvia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input checked="" type="checkbox"/> Other Specify:	Food supplements
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Lithuania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Luxemburg	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Malta	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Norway	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Poland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Portugal	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Romania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Slovak Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Combinations only
Slovenia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
Spain	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No product
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	no response
United Kingdom	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	

¹⁶ This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

¹⁷ Not mandatory field

ASSESSMENT REPORT

BASED ON ARTICLE 16D(1), ARTICLE 16F AND ARTICLE 16H OF DIRECTIVE 2001/83/EC AS AMENDED (TRADITIONAL USE>

Herbal substance(s) (binomial scientific name of the plant, including plant part)	Syzygium aromaticum, flos, Szygium aromaticum, aetheroleum
Herbal preparation(s)	
Pharmaceutical forms ¹⁸	
Rapporteur ¹⁹	Austria
Assessor(s) ²⁰	Reinhard Länger, Birgit Hochenegg

3.2.1. Introduction

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

Herbal substance(s)²¹:

According to the European Pharmacopoeia Caryophylli flos consists of the dried flower buds of *Syzygium aromaticum* (L.) Merrill. et L.M. Perry which were dried until they become reddish brown. They contain not less than 150 ml/kg essential oil.

Caryophylli floris aetheroleum is according to the European Pharmacopoeia obtained by steam distillation from the dried flower buds of *Syzygium aromaticum* (L.) Merrill et L.M. Perry.

Constituents (according to Blaschek et al 2008):

Essential oil: 15 – 17 %, other sources 16 – 21 %, three main components: eugenol (70 – 90 %), eugenyl acetate (- 17 %), caryophyllene (5 – 12 %), main β -caryophyllene, account for 99% of the oil.

Monoterpenes: eugenol (C₁₀H₁₂O₂, Mr164,2) is the chief constituent of caryophylli floris aetheroleum, making up 60 – 95%, moreover it contains 2 – 15% eugenyl acetate (C₁₂H₁₄O₃, Mr206,2), chavicol, (Z)- und (E)-isoeugenol, benzylacetate, α - und β -pinen, limonene

Sesquiterpenes: 5 to 10% β -caryophyllene and the accompanying substances α -ylangene, γ - und α -caryophyllene (= humulene), caryophyllene epoxid, caryophyllenoxide, caryophylla-3(12),7(13)-dien-6 α -ol und caryophylla-3(12),6-dien-4-ol [39] sowie 4,4-dimethyltricyclo[6.3.2.0^{2.5}]trideca-8-en-1-ol, caryophylla-4(12),8(13)-dien-5 β -ol, caryophylla-3,8(13)-dien-5 α -ol und caryophylla-3,8(13)-dien-5 β -ol, α -copaen, α -cubeben, farnesol

Aldehyde: benzaldehyde, m-methoxybenzaldehyde

Alcohols: benzyl alcohol

Ketone: the flavorings heptan-2-one (= methyl-n-amylketone) und octan-2-one (= methylheptylketone)

Hydrocarbons: naphthalene

¹⁸ Pharmaceutical form only according to EDQM standard term

¹⁹ Name of HMPC/MLWP member (not Member State)

²⁰ To be deleted at publication stage, unless Rapporteur requests to keep the name(s)

⁶ According to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMA/HMPC/182320/2005 Rev.2) and the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use (EMA/HMPC/182352/2005 Rev.2)

Acetophenonderivate: 2,6-dihydroxy-4-methoxyacetophenone, methylxanthoxylin

Flavone: quercetin, kaempferol, kaempferid, rhamnetin, kaempferol-3-*O*- β -D-glucoside, quercetin-3-*O*- β -D-glucoside, quercetin-3-*O*- β -D-galactoside, quercetin-3,4'-*O*- β -D-diglucoside

Tannins: ellagitannins, including eugeniin

Penol acids: gallic- and ellagic acid, 3- und 4-caffeoyl-, 3-*p*-cumaroyl- and 3-feruloylchina acid, ferulic, *p*-hydroxybenzoic, coffee, salicylic, syringa, vanillic, gentisic, protocatechuic and *p*-coumaric acid

Triterpene: oleanolic acid, crataegolic acid

Phytosterols: β -sitosterol, stigmasterol, campesterol

Sugars: glucose, xylose, arabinose

Herbal preparation(s):

No references could be found on the medicinal use of *Caryophylli flos* in authorised or registered products.

Herbal preparations: *Caryophylli aetheroleum*

The essential oil is traditionally used since decades for the temporary relief of toothache and as mouthwash for disinfection of the oral cavity (e.g. Frerichs et al 1938). The essential oil is the only active substance of several authorised medicinal products in UK. Although none of these products fulfils the criterion of 30 years of medicinal use (authorisation dates back to 1988), the evidence on traditional medicinal use is given by a large number of publications providing consistent information.

Combinations of herbal substance(s) and/or herbal preparation(s)²²

The use of *Caryophylli flos*, *Caryophylli foris aetheroleum* and its preparations is not described with other herbal substances or herbal preparations. This monograph refers only to *Caryophylli flos*, *Caryophylli foris aetheroleum*.

Vitamin(s)²³

Not applicable.

Mineral(s)¹⁰

Not applicable.

Information about products on the market in the Member States

Austria: only combination products

Belgium: food supplements and combination products

²² According to the 'Guideline on the clinical assessment of fixed combinations of herbal substances/herbal preparations' (EMA/HMPC/166326/2005)

²³ Only applicable to traditional use

Latvia: food supplements and combination products

Slovak Republic: only combination products

Spain: No product

United Kingdom: Several products containing Caryophylli aetheroleum are authorised.

Search and assessment methodology

Search terms: *Syzygium aromaticum*, Gewürznelke, Caryophylli flos, eugenol

Databases: Pubmed, Medline and Toxnet.

Libraries: University Vienna, center of pharmacy; Medical University Vienna, central library.

3.2.2. Historical data on medicinal use

Information on period of medicinal use in the Community

The medicinal use of Caryophylli flos can be traced in literature back to the 13th century (cited in Benedum et al 2006), it is also mentioned by Matthiolus and Lonicerus in the 17th century (cited in Benedum et al 2006).

In fact Caryophylli flos has been in therapeutic use for many decades.

Therefore for Caryophylli flos a period of at least 30 years in medicinal use as requested by Directive 2004/24 EC for qualification as a traditional herbal medicinal product is easily fulfilled.

The medicinal use of Caryophylli floris aetheroleum can be traced in literature back to the 15th century (according to Gildemeister & Hoffmann 1899), it is also mentioned by Schröder and Vietz in the 17th and 18th century (cited in Benedum et al 2006).

In fact Caryophylli floris aetheroleum has been in therapeutic use for many decades.

Therefore for Caryophylli floris aetheroleum a period of at least 30 years in medicinal use as requested by Directive 2004/24 EC for qualification as a traditional herbal medicinal product is easily fulfilled.

Documented use in authorised/registered herbal medicinal products with clove essential oil as the only active ingredient:

UK: since 1988

The type of tradition: European.

Information on traditional/current indications and specified substances/preparations

Caryophylli flos is traditionally used as spice, for example for gingerbread flavouring. Many spice blends, including curry contain powdered cloves; most bitter liqueurs contain clove macerates (Blaschek et al 2008).

The Commission E monographs indicate the use of cloves in inflammatory changes of the oral and pharyngeal mucosa and in dentistry, for topical anaesthesia (Blumenthal et al 1998). Clove has been traditionally used in dyspeptic complaints, flatulence and diarrhoea as a decoction. With foul-smelling breath chew a clove over time (Blaschek et al 2008).

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

Caryophylli floris aetheroleum is traditionally used for external or local applications for the treatment of toothache, and minor infections of the mouth and skin, dressing of minor wounds, sore throats and coughs associated with the common cold, Caryophylli floris aetheroleum or eugenol alone mixed with filling material (zinc oxide) as temporary filling, myalgia, rheumatic complaints, insect bites, flatulent colic, nausea (Blaschek et al 2008, WHO Monographs 2002, Koch 1953, Dingermann et al 2004, Barnes et al 2002, Frerichs et al 1938).

Use as a repellent: Especially suitable for the prevention of mosquito bites.
Cosmetics: Clove oil is used for perfuming of soaps, toothpastes and oral hygiene.
Household / spices: To flavour alcoholic beverages (type bitters and vermouth), confectionery, bakery products and sauces (Blaschek et al 2008).

Indications of authorised/registered herbal medicinal products:
UK: For the temporary relief of toothache due to a dental cavity

Proposed indication: For the temporary relief of toothache due to a dental cavity and for the treatment of minor inflammations of the symptomatic treatment of minor inflammations in the mouth or the throat.
Caryophylli floris aetheroleum is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.
The high content of eugenol makes the medicinal use in the proposed indication plausible.

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

Posology

The herbal substance is not used in defined products; no medicinal product can be traced during the last decades. The essential oil is contained in mouth washes; according to the German Commission E herbal medicinal products should contain the essential oil in a strength of 1-5% (Blumenthal et al 1998).
For the use in children between 1 and 4 years of age a strength of 1 – 2%, for children older than 4 years and adolescents a strength of 1 – 5% are suggested (Dorsch et al 2002).

Duration of use

Not applicable.

Method of administration

The oromucosal and oral administration are the only ways of administration (Blaschek et al 2008).

3.2.3. Non-Clinical Data

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

Effects of Caryophylli flos: antiseptic, antibacterial, antifungal, antiviral, local anaesthetic and spasmolytic effects are attributed to the drug. This information is only partially covered by experimental work (Blaschek et al 2008).

Antimicrobial effects: The additive of 1 g clove powder to 9 ml culture medium inhibited the growth of *Aspergillus flavus*, *A. ochraceus* and *A. versicolor* totally (Hitokoto et al 1980).

A MeOH extract from the dried buds of *S. aromaticum* L. (clove) demonstrated preferential antimicrobial activity against the periodontal pathogens *Prevotella intermedia* and *Porphyromonas gingivalis* with MICs of 156 and 625 µg/ml (Cai & Wu 1996).

Synergism between 13 antimicrobial drugs and 8 plant extracts against *Staphylococcus aureus* strains. *S. aromaticum* L. showed the highest activity. The MIC 90% range was 0,36 mg/ml for clove. Clove extract presented synergism with 11 of 13 drugs so the synergistic capacity was promising (Betoni et al 2006).

Antiviral effect: An aqueous extract of clove combined with acyclovir showed a stronger anti-HSV-1 activity as treatment with acyclovir alone. No signs of toxicity of the herbal preparation were observed after administration of 250 mg/kg BW for 7 days (Kurokawa et al 1995).

Anticarcinogenic effects: DMBA-croton oil treated mice had a visible rough granular surface on the shaved skin with varying degrees of erythema and sometimes with white plaque like lesion. Treatment

with aqueous infusion of clove delayed the onset of papillomas in treated groups. Treatment was most effective in the group which receive the clove infusion orally. At the dose of 100 µl the onset of papilloma was delayed by two weeks. 200 µl clove infusion also delayed onset of papilloma but not to the extent seen with 100 µl. 50 µl of clove infusion were not effective (Banerjee & Das 2005).

The effect of clove aqueous infusion was very pronounced ($p < 0,01$) on the incidence of carcinoma in situ (CIS). The infusion was administered at a dose of 100 µl/mouse/day. While 70% of Benzopyrene-exposed animals (Newborn Strain A mice) had CIS, after treatment with clove infusion only 10% animals showed CIS indicating 85,71% inhibition after such treatment. Incidence of hyperplasia and dysplasia evident in the carcinogen control group, were effectively reduced after treatment with clove infusion. Significant reduction in the number of proliferating cells and an increased number of apoptotic cells was also noted in these Benzopyrene-induced lung lesions following clove treatment (Banjeree et al 2006).

Assessor's comment: These observations support the idea that spices possess anticarcinogenic properties and may contribute to maintain health.

Molluscicidal effect: The snail *Lymnaea acuminata* is the intermediate host for *Fasciola*-species causing fascioliasis in northern India. The toxicity of flower-bud powder of *Syzygium aromaticum* L. and its organic solvent extracted fractions against the snail *Lymnaea acuminata* were time and concentration dependent. The LC50 of flower-bud powder of *Syzygium aromaticum* L. at 24 h was 172,75 mg/l and at 96 h 51,98 mg/l, respectively. Ethanol extract was more toxic than other organic extracts. The 24 h LC50 of ethanol extract of *S. aromaticum* flower-bud powder against *L. acuminata* was 83,53 mg/l. The 24 h LC50 of the column purified fractions of *S. aromaticum* flower-bud powder was 20,73 mg/l; 96 h LC50 7,87 mg/l. 24 h LC50 of eugenol was 11.03 mg/l (Kumar & Singh 2006).

Assessor's comment: These results clearly indicate that flower-bud powder of *Syzygium aromaticum* L. could be an important source of molluscicides.

Sexual behaviour:

The effects of an ethanolic extract of clove (DER app. 10:1, ethanol 50%) were evaluated and compared with the standard drug Sildenafil citrate. The extract was administered orally to adult male Swiss mice at a dosage of 500mg/kg. Clove ethanolic extract and the standard drug Sildenafil displayed excessive mounting behaviour 1 hour after treatment as well as 3 hours after treatment (Tajuddin et al 2003).

The extract increased the mounting frequency, intromission frequency, ejaculatory latency in first series and decreased the mounting latency, intromission latency in a significant manner. At doses of 100 mg/kg no significant change of the mounting frequency was observed. No signs of toxicity were observed (Tajuddin et al 2004).

Other effects: Male, 7 to 8 weeks old swiss-albino mice were given over 10, 20 or 30 days feed with 0,5%, 1% and 2% (m/m) clove powder. The content of acid-soluble, free SH-groups in the liver homogenate increased significantly dose- and time-dependent compared to untreated controls. After 30 days 27, 79 or 30.01 or 33,10 µmol/g tissue (control 19,89 µg/g; $p < 0,005$) were found. The formation of malondialdehyde was reduced after γ -irradiation: 30 days, 1,90 ($p < 0,01$), 1,01 or 0,89 ($p < 0,0005$) nmol/mg protein; control 2,29 nmol/mg protein. The Glutathion-S-Transferase activity and the cytochrome- b₅-content was in all groups (with the exception 0, 5%, 10 days) increased significantly compared with controls ($p < 0,0005$ to $p < 0,01$). The cytochrome-P₄₅₀-content fell significantly in all dose groups after 30 days. The activity of arylhydrocarbonhydroxylase was unaffected. A possible protective effect of the herbal substance against chemical pollutants is discussed (Kumari 1991).

A methanol extract from cloves induced the differentiation of M1-cells (myelotic leukaemia of mouse) in macrophage in vitro. The fractionation of the extract yielded oleanolic acid and crategolic acid as active ingredients, which induce in a concentration of 5×10^{-5} or 2×10^{-5} M a differentiation. Oleanolic acid resulted in a concentration of 5×10^{-6} M also in a differentiation of HL-60 cells (promyelotic human leukaemia) while the effect of crategolic acid was overlaid by cytotoxic effects. The authors investigated

by means of derivatives structure-activity-relationships. There is a lack on further pharmacological information (Umehara et al 1992)

Clove infusion reduced the COX-2 level on the 17th and 26th weeks but not on the 8th week (Banjeree et al 2006).

Anti-Hepatitis C Virus Protease-activity was caused by *Syzygium aromaticum*. The methanol extract exhibited significant inhibitory activity ($\geq 90\%$ inhibition). The IC₅₀ was 33, 0 $\mu\text{g/ml}$ (Hussein et al 2000).

The toxic effect of cloves on *Culex pipiens* larvae, the common European mosquito, was investigated by El Hag et al (1999). The LC₅₀ for the methanol extract was 824, 7 ppm (assay time: 6 days) and the LC₅₀ for the ethanol extract was 921,3 ppm (assay time: 6 days). The highest mortality (70%) was obtained in the 1000ppm concentration after 10 days.

Antithrombotic effects: Two different polysaccharides with rhamnogalacturan backbone and arabinan side chain were isolated which exhibit antithrombotic activity. After intravenous application of the low molecular weight polysaccharide (MW 34.000) in doses up to 1000 mg/kg BW in mice no signs of acute toxicity were observed, while the high molecular weight polysaccharide (MW 103.000) exhibited approximately half the toxicity of heparin (LD₅₀ 322 mg/kg) (Lee et al 2001).

Antiallergic effects: Kim et al (1998) investigated the effect of a hot water extract (DER app. 14:1) of clove on the immediate hypersensitivity in rats. The extract inhibited the compound 48/80-induced systemic anaphylaxis in rats with an IC₅₀ of 31.25 mg/kg when administered intraperitoneally. The extract also inhibited the local immunoglobulin E-mediated passive cutaneous anaphylactic reaction (IC₅₀ = 17.78mg/kg, i. v., IC₅₀ = 19.81 mg/kg, p. o.). The extract also inhibited dose-dependently the induced histamine release from rat peritoneal mast cells.

Effect on NO-formation: A decoction (0.1%) of clove reduced NO levels by 57.2%, in comparison with the control value at a concentration of 250 $\mu\text{g/ml}$. The scavenging effects were concentration-dependent (Yokozawa et al 2000).

Antioxidative effects: A decoction (10%) of clove exhibited antioxidative effects on the lipid peroxidation and protein oxidative modification of mice brain homogenate produced by copper in vitro (Toda 2001, Toda 2003).

Effects of *Caryophylli floris aetheroleum*:

Due to a high content of eugenol in *Caryophylli floris aetheroleum*, the effects of eugenol are claimed for the essential oil as such.

Eugenol acts spasmolytic, choleric, local anaesthetic and it inhibits the motility, as well as a spasmolytic and local anaesthetic effect of eugenyl acetate (Gracza 1980 cited in Blaschek et al 2008).

Analgesic effects: Patch-clamp experiments showed that eugenol reversibly activates calcium ion channels and chloride ion channels in dorsal root ganglion cells from rats. Eugenol concentrations used were from 0,125 to 1 mmol/l. These effects may be the responsible for the analgesic activity (Gruenwald et al 2004). Caryophyllene oxide and eugenol as Ca⁺⁺-channel-blockers (Sensch et al 1993 cited in Blaschek et al 2008).

Sodium and Calcium channels act as molecular targets for eugenol for its analgesic effect. Eugenol inhibits ATP-induced P2X currents in trigeminal ganglion neurons, which contributes to the analgesic effect (Li et al 2008).

Intrathecal treatment of mice with eugenol (12.5 to 50 μg) for 24 hours dose-dependently inhibited the formalin-induced nociceptive response. Capsazepine shifted the dose-response curves in parallel to the right. Eugenol may exert its antinociceptive effect via the capsaicin receptor located on sensory terminals in the spinal cord. These results are novel findings indicating that eugenol act as capsaicin-like substance (Ohkubo & Shibata 1997).

Antiinflammatory effects: Eugenol inhibited the NO production in a dose-dependent manner in the RAW264,7 cells treated with 1 µg/ml Lipopolysaccharid for 24 h. Isoeugenol was more effective. LPS-dependent expression of COX-2 was also inhibited by isoeugenol and less effectively by eugenol (Li et al 2006).

Anticarcinogenic effects: The sesquiterpenes β-caryophyllene, β-caryophyllene oxide, alpha-humulene, alpha-humulene epoxide and eugenol induced the detoxifying enzyme glutathione S-transferase in the mouse liver and intestine. These compounds show promise as potential anticarcinogenic agents. Mice got 20 mg of isolated sesquiterpenes once every 2 days (Zheng et al 1992).

Eugenol showed chemo preventive effects. Eugenol, but not its isomer isoeugenol was found to be a potent inhibitor of melanoma cell proliferation. It inhibits the growth of melanoma cells in culture (50% inhibition by 0.5 µM). Eugenol causes significant tumour growth delay, decrease of tumour size and prevents tumour metastasis in mice (125 mg/kg) (Ghosh et al 2005).

Antimicrobial effects: The majority of publications on pharmacological effects of clove or clove essential oil deal with the antimicrobial effects. Only some selected references are cited below.

0, 4% clove oil in 63% sugar syrup inactivated after 2 – 7 min *Candida albicans*, *Clostridium perfringens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* und *Staphylococcus aureus*. The effect was not affected by addition of serum. Added sugar is not needed for an effect; however, it stabilizes the dispersion of the essential oil (Briozzo et al 1989).

The effect of 0 – 300 ppm clove oil on the growth and synthesis of aflatoxins from *Aspergillus parasiticus* was studied in submerged culture. 0 – 250 ppm made a slower growth, but had no influence on the mycelweight after 21 days. 300 ppm completely inhibited the growth. The aflatoxinproduction was dose-dependent delayed. With 250 ppm the maximum aflatoxinproduction of the control was not achieved even after 3 weeks (Bullerman et al 1977).

In an investigation of various aromatic waters, clove oil – water had no sustainable growth-inhibiting effect in load with *pseudomonas*. Clove oil exhibited in the serial dilution test and agar diffusion test only a weak antimicrobial effect. The MHC in the serial dilution test was in the case of *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* for each 1:20, for *Mycobacterium phlei* 1:640 and for *Staphylococcus aureus* 1:160 or 1:320. The minimal fungicidal dilution was 1:320 in case of *Aspergillus niger*, for *Penicillium chrysogenum* 1:40 and for *Mucor*, *Rhizopus* and *Candida albicans* for each 1:20. The results in agar diffusion test differ in part from that in the serial dilution test (Yousef & Tawil 1980).

Ali et al (2005) found that eugenol inhibits the growth of 30 *Helicobacter pylori* strains tested, at a concentration of 2µ/ml after 9 and 12 hours of incubation. A lower pH-value increased the activity. The bacteria did not develop any resistance even after 10 passages grown at sub-inhibitory concentrations. The authors conclude that eugenol may prevent the growth of *H. pylori*.

The antifungal acitivity of *Syzygium aromaticum* essential oil against *Aspergillus section Flavi* was evaluated in sterile maize grain. The effect of the essential oil added to maize grains on growth rate, lag phase, and aflatoxin B₁ (AFB₁) accumulation of *Aspergillus section Flavi* were evaluated at different water activity conditions (0,982; 0,955; and 0,90). The essential oil had an inhibitory effect on *Aspergillus section Flavi* growth rate; the efficacy depended mainly on the water activity and concentration. Clove essential oil showed a considerable inhibitory effect on the AFB₁ accumulation. When the water activity was 0,982, the AFB₁ inhibition percentage for all aflatoxigenic strains exceeded 98% at all clove essential oil concentrations (Bluma & Etcheverry 2008).

Dorman & Deans (2000) tested the antibacterial activity of the essential oil of *S. aromaticum* in 25 bacteria The results suggest that the essential oil is equally effective against both Gram-positive and Gram-negative microorganisms. A different sensitivity of the bacteria tested was observed.

Eugenol significantly reduced the number of colony forming units sampled from the oral cavity or immunosuppressed rats treated for 8 days. Eugenol was used in a concentration of 24 mM (= double MIC) in agar solution. As positive control served Nystatin in a concentration of 58 μ M (= tenfold MIC). Eugenol and nystatin gave similar results. Only few zones were occupied by hyphae, while under nystatin hyphae were found in the folds of the tongue mucosa (Chami et al 2004). There was a significant reduction of colony counts in a prophylactic and a treatment approach in case of vaginal candidiasis in an immunosuppressed rat model. The rats received 10 mg/kg/day Eugenol via an intravaginal route (Chami et al 2004).

Lee et al (2007) evaluated the antifungal effect of eugenol against *Microsporum gypseum*. Eugenol was adjusted to 10% concentration with a base of Vaseline petroleum jelly and was applied topically to the infected skin lesions daily for 3 weeks. Eugenol was clinically active.

Saini et al (2009) investigated the effect of orally administered essential oil on respiratory tract infections with *Klebsiella pneumoniae* in rats. The daily oral supplementation was 0.5 ml of a 1% w/v solution. The comparison of short term (15 days) and long term (30 days) treatment resulted in a significantly lower bacterial load in the lungs of mice fed clove oil for 30 days. The authors stated also a significant decrease of bacterial colonization already after 15 days.

The composition and antifungal activity of clove essential oil were tested by Pinto et al (2009). MICs, determined according to Clinical and Laboratory Standards Institute protocols, and minimum fungicidal concentration were used to evaluate the antifungal activity of the clove oil and its main component, eugenol, against *Candida*, *Aspergillus* and dermatophyte clinical and American Type Culture Collection strains. The essential oil and eugenol showed inhibitory activity against all the tested strains. Propidium iodide rapidly penetrated the majority of the yeast cells when the cells were treated with concentrations just over the MICs. Therefore the fungicidal effect may result from extensive lesions of the cell membrane.

Clove oil and eugenol also caused a considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component. Germ tube formation by *Candida albicans* was completely or almost completely inhibited by the essential oil and eugenol concentrations below the MIC values. The authors conclude that the results indicate that clove oil and eugenol have considerable antifungal activity against clinically relevant fungi, including fluconazole-resistant strains.

Antiviral effect: Eugenol, which was found at a concentration of 0.1% in cloves, inhibited in vitro in FL-cell cultures the replication of herpes simplex virus at a concentration of 10 μ g/ml (Takechi M & Tanaka Y 1981).

Kurokawa et al (1998) studied the effects of eugenol on Herpes simplex virus -1. The effective concentration for 50% plaque reduction for HSV-1 on Vero cells was 5.0 μ g/ml, which is approximately 14 fold lower than the 50% cytotoxic concentration. The viral DNA synthesis was found to be one of the major target sites of the inhibitory action.

For eugenol no significant antiviral activity against herpes simplex virus type 1 was found (Astani et al 2009).

Spasmolytic effect: A saturated aqueous solution of clove oil was active in vitro on isolated organs against various spasmogenes: rat/duodenum/acetylcholine: 20 to 40% inhibition; rat/duodenum/bariumchlorid: 40 to 60% inhibition; guinea-pig/ileum/histamine: >60% inhibition; rabbit/jejunum/nicotin: >60% inhibition. No further details on the methodology are available (Debelmas & Rochat 1967 cited in Blaschek et al 2008).

Clove oil antagonized in vitro the carbachol-induced spasm of the muscles of the trachea of the guinea-pig and the electrically stimulated contraction of the longitudinal muscles of the ileum of the guinea-pig. The

EC50 was 3,8 mg/ml (trachea; isoprenaline EC50 = 3,9 nmol/l) or 6,8 mg/ml (ileum; papaverine EC50 = 3,7 μ mol/l) (Reiter M & Brandt W 1985).

Eugenol relaxes the rabbit thoracic aorta while suppressing the Ca²⁺ sensitivity and both the uptake and extrusion mechanisms for Ca²⁺ (Nishijima et al 1999).

Effect on coagulation: Clove oil inhibited in vitro the platelet aggregation which was induced by arachidonic acid, epinephrine and collagen. The formation of thromboxane B₂ induced by arachidonic acid was inhibited in intact and in lysed platelet preparations. The effect, which exceeds the in vitro effect of acetylsalicylic acid might be attributed to eugenol and eugenyl acetate. The combination of these compounds inhibits the platelet aggregation in a superadditive manner (Srivastava & Malhotra 1991, Srivastava 1993, Srivastava & Justesen 1987, Srivastava 1990 cited in Blaschek et al 2008, Srivastava 1993).

Effect on prostaglandin synthesis: At in-vitro-preparations from the seed bubble of sheep, inhibits the additive of 37 μ M clove oil (based on average molecular weight of 200) the prostaglandin synthesis from 1-¹⁴C-arachidonic acid compared to a control without the essential oil by 84,1%. IC₅₀ of eugenol 11,0 μ M, of indomethacin 1,2 μ M (Wagner et al 1986). Eugenol and its derivatives are inhibitors of LOX-5 and COX-2 (Hübner 2008).

Sedative effect: Wagner & Sprinkmeyer (1973) investigated the sedative effect of clove essential oil. Mice received 1 to 100 mg/kg KG p. o. The motility in the photocell cage was compared with the results of the day before (without treatment). The authors observed a not dose-dependent reduction of motility.

Assessor's comment: A statistical evaluation is lacking. The investigation is due to significant deficiencies not suitable for a detectable sedative effect.

Antiprotozoal effects: Clove oil inactivated in vitro *Trichomonas vaginalis* in a dose-and time-depending manner. After addition of 4, 2, 1, 0,5 and 0,25 mg/ml to the culture medium no surviving *Trichomonads* were detectable after an incubation period of 5 min to 8 hours. Concentrations of 0,1 and 0,05 mg/ml were not effective. With eugenol comparable effects were achieved. With 4 – 0,05 mg/ml of the reference substance metronidazol the effect was achieved after 30 min to 2 h (Salem 1980 cited in Blaschek et al 2008).

Treatment of epimastigotes of *Trypanosoma cruzi* with different concentrations of clove essential oil resulted in a dose-dependent growth inhibition, with IC₅₀/24 h of about 99,5 μ g/ml; IC₅₀/24 h values obtained after treatment of bloodstream trypomastigotes were about 57,5 μ g/ml. The values obtained for epimastigotes treated with eugenol were 246 μ g/ml, while treatment of bloodstream trypomastigotes resulted in IC₅₀/24 h values of 76 μ g/ml for eugenol (Santoro et al 2007).

Effect as repellent: In study by Eamsobhana et al (2009) commercially produced essential oils of 13 plant species and ethanol (control) were tested for repellent activity against host-seeking chiggers of *Leptotrombidium imphalum*. Dilutions of each essential oil were prepared in absolute ethanol. Clove essential oil exhibited 100% repellent activity at 5% concentration. Eugenol as repellent (Hassanali et al 1990 cited in Blaschek et al 2008).

Antimutagenic activity: Miyazawa & Hisama (2003) identified dehydrodieugenol and trans-coniferyl aldehyde as the antimutagenic compounds in clove. These compounds showed suppressive effects on umu gene expression of the SOS response in *S. typhimurium* TA1535/pSK10002 against furylfuramide, 4NQO, and MNNG, which do not require liver-metabolizing enzymes, and AFB₁ and Trp-P-1, which require liver-metabolizing enzymes and UV irradiation. Dehydrodieugenol had stronger suppressive potencies.

Anti-genotoxicity: Anti-genotoxic effects of eugenol were assessed in the mouse bone marrow micronucleus test by Abraham (2001). The test doses of eugenol were administered to mice by gavage 2 and 20 h before exposure to the genotoxin. A pre-treatment with 50-500 mg / kg BW eugenol resulted in significant reductions with cyclophosphamide, procarbazine, methylnitronitrosoguanidine and urethane. The administration of eugenol alone did not exert genotoxicity.

Cytotoxicity: An in-vitro study demonstrates cytotoxic properties of both the oil and eugenol, towards human fibroblasts and endothelial cells. Clove oil was found to be highly cytotoxic at concentrations as low as 0.03% (v/v) with up to 73% of this effect attributable to eugenol. β -Caryophyllene did not exhibit any cytotoxic activity, indicating that other cytotoxic components may also exist within the essential oil. The viability of all cell types dropped by 60 – 90% when the concentration of the oil was increased from 0.01% to 0.03% (Prashar et al 2006).

High doses (0.05% clove oil; 2.50mM eugenol) of the essential oil and its components into culture media already markedly increased the percentage of both necrotic and apoptotic cells after 1 h (clove oil: 18,04%; eugenol: 21,64%). The medium doses (0.01% clove oil; 0.52mM eugenol) did not cause significant damage to the Caco-2 population after 1 h culture when compared with control (Fabian et al 2006).

Use of eugenol in dentistry

Eugenol can be part of temporary pulp fillings in dentistry. Eugenol is mixed with zinc oxide giving a paste which hardens quickly when coming into contact with saliva. This special application is not within the scope of a Community monograph and will therefore not be further discussed.

Other effects:

Hepatoprotective Effects: Eugenol may protect the liver from damage by certain chemicals, including iron overload. The mechanism may involve eugenol acting both as an antioxidant to prevent lipid peroxidation and by scavenging free radicals by conjugating with glutathione. In an animal study, eugenol reduced the hepatic injury caused by iron overload. Eugenol lowered liver lipid peroxidation by 38% and serum lipid peroxidation by 30% in iron-treated rats (Gruenwald et al 2004).

Induction of Glutathion-S-Transferase by eugenol and caryophyllene (A&B) and caryophyllene oxide (A&B) (Patel et al 1977 cited in Blaschek et al 2008).

Clove essential oil increased the total white blood cell count and enhanced the delayed-type hypersensitivity response in mice. Moreover, it restored cellular and humoral immune responses in cyclophosphamide-immunosuppressed mice in a dose-dependent manner. The immunostimulatory activity found in mice treated with clove essential oil is due to improvement in humor- and cell-mediated immune response mechanisms (Carrasco et al 2009).

Eugenol attenuates the reduction of dopamin. Eugenol administration 3 days before and 7 more days following one intracerebroventricular injection of 6- hydroxydopamine prevented the reduction of striatal dopamine and its metabolites. This effect suggests it possible usefulness for the treatment of Morbus Parkinson (Kabuto et al 2007).

Eugenol depressed cell respiration in homogenates of human dental pulp and in mouse fibroblast monolayers. The authors conclude that the irritant effect of zinc oxide eugenol when applied directly to soft tissue is due to the fact that concentrations of eugenol are achieved which are sufficient to inhibit respiration and thus kills cells (Hume 1984).

Molluscicidal activity: Treatment with 20% and 60% of the 96 h LC₅₀ of eugenol caused in the test animals of the snail *Lymnaea acuminata* significant inhibition of the alkaline phosphatase and acetylcholinesterase activities (Kumar et al 2009).

Allen & Cornforth (2009) demonstrated the iron chelating ability of eugenol. In presence of iron, Type I antioxidants like eugenol had a significant prooxidant effect.

The aim of the study by Kahn et al (2009) was to evaluate quorum sensing (QS = mechanism by which bacterial population measures its cell density) inhibitory activity of plant essential oils using strains of *Chromobacterium violaceum* (CV12472 and CV026) and *Pseudomonas aeruginosa* (PAO1). Significant inhibition of pigment production was detected in clove oil with 19 and 17 mm zone of pigment inhibition against CV12472 and CV026 strains. Clove oil, at lower concentration (2 µl) showed no activity, but at higher concentration (20 µl) antibacterial activity was observed along with anti-QS activity (zone of inhibition 21 mm). Eugenol does not seem to be responsible for these effects.

Eugenol fulfills all points of a narcotic for commercial fish. Clove oil is allowed as food additive and therefore an administration to food-producing animals is possible. The allowed daily intake for the United States is reported with 2.5 mg/kg (Oettinger 2003).

Clove oil is active against the eggs and second-stage juveniles of the parasitic nematode *Meloidogyne incognita* (Meyer et al 2008).

Mild hypertension has resulted in dogs after receiving 0,05 ml of eugenol (Gruenwald et al 2004).

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

No specific data are available on *Caryophylli flos* or *Caryophylli floris aetheroleum*.

In case of systemic treatment an effect of phenolic compounds is only expected at higher doses because of a rapid metabolism (Wagner 2003).

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

Toxicity of *Caryophylli flos*:

Acute Toxicity:

The acute toxicity of a decoction of clove was studied in 30 overnight fasted mice. Doses of 100-520 mg/kg BW were administered intraperitoneally, larger doses of 500-5000 mg/kg BW by oral gavage. The animals were observed for respiratory, GIT, CNS symptoms, behavioural patterns and mortality. The only toxic manifestation was abdominal cramps.

The LD50 was interpolated as 263 mg/kg (i. p.) and 2500 mg/kg (oral) (Agbaje et al 2009).

Tajuddin et al (2003) studied the acute toxicity of an ethanolic extract (DER app. 10:1, ethanol 50%). 6 mice received 500 mg/kg extract p.o. No signs of mortality or gross behavioural changes were observed.

Subchronic toxicity:

Swiss albino mice received for 10, 20 and 30 days 0.5%, 1% and 2% w/w clove powder in the diet. Enhanced Glutathion-S-transferase, cytochrome b5 and sulphhydryl enzymes levels were observed in all the treatment groups, excepting those maintained on a 0.5% diet for 10 days. A significant reduction in CYP-450 and malondialdehyde levels was observed in all groups at 30 days duration (Kumari 1991).

After 90 days of administration of a decoction of clove at doses of 300 mg/kg and 700 mg/kg in rats significant alterations in liver enzymes and haematological parameters were observed. Even in the lower dose histopathological modifications could be found in body organs. The authors conclude that a prolonged use of decoctions of clove should be avoided (Agbaje et al 2009).

Mutagenicity:

The dry residue of aqueous and methanolic extracts showed mutagenic effects in the rec assay in *Bacillus subtilis* mutagenic. The mutagenic activity in the Ames test on *Salmonella typhimurium* TA98 and TA100 was not assessable due to the antimicrobial action (Morimoto et al 1982).

An anti-microbial effect from drug quantity of 50 mg / plate in another work, also makes the assessment of the mutagenicity of extracts of clove on TA98 and TA100 impossible (Opdyke 1975 cited in Blaschek et al 2008).

After administration of a decoction (1:100) of cloves to *Drosophila* no genotoxic effects were observed (Schulz & Herrmann 1980 cited in Blaschek et al 2008).

An in vivo bone marrow micronucleus assay demonstrated that the administration of 0.5% and 2% of clove in the diet of mice for 10 days neither significantly induced micronuclei nor could effectively modulate the 7, 12-dimethylbenz[a]anthracene genotoxicity in mice (Kumari 1991).

Reproduction toxicity:

Data from *Caryophylli flos* are not available. However, the herbal substance contains oleanolic acid. For oleanolic acid isolated from *Syzygium jambos* flowers a possible anti-estrogenic effect is documented (Rajasekaran et al 1988).

Rats received orally 15 mg /kg BW or 30 mg/kg BW oleanolic acid daily over a period of 60 days. This dosage is equivalent to app. 0.8 to 3 g clove per kg BW. A histological examination of the testes showed a dose-dependent reduction in the number of spermatides and spermatocytes of 2.69 (control) to 1.73 and 0.93. The number of fertilized females was reduced from 20/20 (control) to 7 / 20 or 2 / 20. The number of implants also decreased from 8.80 to 5.43 and 2.55.

Mishra & Singh (2008) investigated a hexane extract in doses of 15-60 mg/kg BW per os in mice over 35 days. The treatment did not induce systemic toxicity at the doses tested. At 15 mg/kg BW the activities of testicular enzymes and serum levels of testosterone were increased. At doses of 30 mg and 60 mg/kg BW these parameters were inhibited. Additionally non-uniform degenerative changes in the seminiferous tubules associated with a decrease in daily sperm production were observed.

Toxicity of *Caryophylli floris* aetheroleum and constituents thereof:

Toxicity:

Essential oil (Blaschek et al 2008):

Rat, p.o., 1.8 – 3.72 g/kg

Rabbit, cutaneous application, 5 g/kg

Eugenol (Blaschek et al 2008):

Rat: p.o. LD50 2.68 g/kg; i.p. LD50 800 mg/kg

Mouse: p.o. LD50 3 g/kg; i.p. LD50 500 mg/kg

Acute Toxicity:

After oral administration of 5000 mg/kg of the essential oil in rats, all animals died within 24 h. The autopsy showed bleeding in the stomach and intestines, and pleural effusion (Blaschek et al 2008).

On the isolated rabbit lung, the addition of 1.0 mM eugenol resulted in edema, as measured by the increase in lung weight and wet/dry weight of the lung. The addition of catalase (1000 U/mL) or dimethylthiourea (30 mM) decreased the response. Dimethylurea, superoxide dismutase or heat inactivated catalase had no influence (McDonald & Heffner 1991).

A p. o. without a single dose of 140 mg / animal resulted in rats within a short time to death. Undiluted clove applied on the dorsal skin of hairless mice did not cause irritation. On intact or shaved rabbit skin

clove essential oil acted under occlusive conditions as a weak irritant. Phototoxic effects were not observed with undiluted clove oil on hairless mice and pigs (Opdyke 1975 cited in Blaschek et al 2008).

Chronic toxicity: Clove essential oil in oral dosages of 35 or 70 mg per animal (rat) over 8 weeks of rats were tolerated without signs of toxicity. Higher doses led to inactivity and weight loss. 105 mg / animal p. o. daily for 2 to 3 week led to serious liver and kidney damage and death of the animals (Opdyke 1975 cited in Blaschek et al 2008).

Mutagenicity, Genotoxicity:

No signs of a mutagenic effect could be observed in the in vitro chromosomal aberration test in fibroblasts from Chinese hamsters at concentrations up to 0.04 mg / mL of clove oil (Ishidate et al 1984).

No evidence of a mutagenic activity could be detected in clove oil (10 to 250 µl) in vitro in *Salmonella typhimurium* TA1530 and G46 without metabolic activation (Litton 1975).

The National Toxicology Program (NTP) performed a mutagenic study on eugenol.. The study finished 1980. Outcome:

AMES-Test (TA1535, TA100, TA98, TA1537 strains with mebabolic activation): negative; Mouse lymphoma: positive, Sister chromatide exchange: positive; Chromosome aberrations: positive; Micronucleus: negative; Drosophila: negative; in vivo sister chromatid exchange: positive; in vivo chromosome aberrations: equivocal. (NTP 1980)

Eugenol was tested for mutagenic activity in the AMES-test using *S. typhimurium* TA 1535, TA 100 and TA 98. For TA 100 and TA 98 strains no mutagenicity was detected, but in case of TA 1535 strain dose-dependent mutagenicity was observed (Swanson et al 1979).

Eugenol induces chromosome aberrations in Chinese hamster lung cells and exerts a cell-transforming activity in Syrian hamster embryo cells (Hikiba et al 2005).

An in vitro chromosome aberration test in fibroblasts of Chinese Hamsters revealed no signs of mutagenicity at concentrations up to 0.04 mg/ml of clove oil (Ishidate et al 1984).

Maralhas et al (2006) demonstrated that eugenol induces chromosome aberrations, including exchanges, in V79 cells, in particular in the presence of rat liver S9 mix, which suggests biotransformation to reactive metabolites. Eugenol induced chromosomal aberrations significantly (3.5% aberrant cells) at 2500 µM, demonstrating cytotoxicity in higher doses. S9 increases the number of aberrant cells to 15% with a high frequency of chromatid exchanges. Additionally an increase in endoreduplicated cells was observed. The authors suggest that eugenol exhibits topoisomerase II inhibiting activity.

Eugenol was also tested by Ellahuene et al (1994) in the mouse bone marrow micronucelus assay. Single doses of 400 and 600 mg/kg eugenol induced a statistically significant increase in the induction of micronucleus-polychromatic erythrocytes compared to the negative control (Ellahuene MF, Pérez-Alzola LP, Orellana-Valdebenito M, Muñoz C & Lafuente-Indo N 1994).

Reproductive and developmental toxicity:

Domaracky et al (2007) investigated the effects of clove essential oil on the growth and development of mouse preimplantation embryos in vivo. The animals received 0.25% essential oil in the commercial diet (=375 mg/kg per day). Treatment with clove essential oil induced a significantly higher percentage of dead cells compared to the control group.

Female CD-1 mice were given from day 6 to day 15 of gestation p. o. 2.2 to 215.0 mg / kg BW of clove oil daily. The foetuses were obtained on day 17th. The use of clove oil had no apparent toxic effect on the implantation and survival of the mother and foetus. The number of malformations of the soft tissues and the skeletal system was not different from that in the control group spontaneously occurring. Same results

were obtained in female Wistar rats following daily p. o. administration of 2.8 to 280 mg / kg BW (6 to 15 of gestation, foetuses at day 20), to golden hamsters after p. o. 1.8 to 177.0 mg / kg BW (6 to 10 of gestation, foetuses at day 14) and in rabbits by 1.72 to 172.0 mg / kg BW (6 to 18 of gestation, foetuses at day 29) achieved (Blaschek et al 2008).

Carcinogenicity:

The carcinogenicity of eugenol evaluated within the US National Toxicology Program. A negative outcome for F344 rats of either sex was found. However, equivocal results were observed in B6C3F1 mice (NTP 1983).

Local toxicity:

Undiluted eugenol (no data on amount of eugenol) was applied to a circumscribed area 3 mm in diameter of rat labial mucosa for one minute. Reaction periods of 15 minutes, 1, 2, 4 and 6 hours were then permitted. Using routine histological procedures for processing the experimental tissues, it was observed that eugenol caused denaturation of cytoplasmatic proteins and loss of staining capacity of epithelium, loss of cell boundaries, swelling and cell necrosis. In addition, vesicle formation, oedema in the corium, and striated muscle dissolution were observed (Kozam & Mantell 1978).

Overall conclusions on non-clinical data

The published data referred to the indications and preparations is very limited, but on the basis of existing data the pharmacological activities support the traditional use of *Caryophylli flos* and *Caryophylli aetheroleum* and preparations thereof in the proposed indication: For the temporary relief of toothache due to a dental cavity and for the treatment of minor inflammations of the symptomatic treatment of minor inflammations in the mouth or the throat.

Since the efficacy of traditional herbal medicinal products is only plausible the safety must be guaranteed. In the case of *Caryophylli aetheroleum* the main component eugenol gives reason for safety concerns. Natural compounds with a similar chemical structure like safrole and methyleugenol are known as genotoxic carcinogens. Available data regarding genotoxicity of eugenol are inconsistent and equivocal. The scientific discussion on risks associated with the oromucosal or oral use of clove or clove essential oil is still at the beginning. A final conclusion cannot be drawn at the moment. In the case that the possible risks overbalance the plausible efficacy the further development of a monograph on clove essential oil has to be cancelled. The safety concerns do not allow the establishment of a Community List Entry.

3.2.4. Clinical Data

Clinical Pharmacology

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

Eugenol caused a 'comfortable feeling' in the 13 female subjects. Alpha 1 of EEG significantly decreased after inhalation. Suppression of alpha 1 indicates the neural activity around the brain regions. There is a possible positive correlation between alpha 1 activity and subjective evaluation (Masago et al 2000).

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

No data available for the entire essential oil.

Eugenol:

The metabolism of eugenol was investigated in male and female healthy volunteers by Fischer et al (1990).

Eugenol was rapidly absorbed and metabolized after oral administration and was almost completely excreted in the urine within 24 h. Unmetabolized eugenol was found in the urine less than 0.1% of the dose. The urine contained conjugates of eugenol and of nine metabolites they were identified as: eugenol, 4-hydroxy-3-methoxyphenyl-propane, cis- and trans-isoeugenol, 3-(4-hydroxy-3-methoxyphenyl)-propylene-1, 2-oxide, 3-(4-hydroxy-3-methoxyphenyl)-propane-1,2-diol, and 3-(4-hydroxy-3-methoxyphenyl)-propionic acid. 95% of the dose was recovered in the urine, most of which (greater than 99%) consisted of phenolic conjugates; 50% of the conjugated metabolites were eugenol-glucuronide and sulphate.

Clinical Efficacy

Dose response studies

There is no data available.

Clinical studies (case studies and clinical trials)

Clinical studies in the proposed indication:

There is no data available.

Other clinical studies:

Central effects: The influence of clove oil on psychometric parameters such as mood, affective reaction, memory and cognitive abilities in 21 male and 51 female probands was studied in a cross-over trial. The concentration in the room air conditioning was corresponding to 0.0057 – 0.0167 g/m³. No differences in the examined parameters could be observed (Wagner & Sprinkmeyer 1973).

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

There is no data available.

Overall conclusions on clinical pharmacology and efficacy

No clinical data are available for Caryophylli flos and Caryophylli aetheroleum in order to support well-established use.

Therefore the medicinal use has to be regarded as traditional.

3.2.5. Clinical Safety/Pharmacovigilance

Overview of toxicological/safety data from clinical trials in humans

There is no data available.

Patient exposure

There is no data available.

Adverse events

Skin and mucosal irritations:

In concentrated form, oil of clove may be irritating to mucosal tissues (Gruenwald et al 2004).

In contrast to this Anton et al (2001) report that there is no skin irritation (undiluted oil) on hairless mice.

Under occlusion clove (undiluted oil) was moderately irritating in rabbits.

With direct application to the exposed pulp, pulp necrosis and inflammation appeared. (Reichl et al 2007)

Allergic effects:

In patients sensitized to Peru balsam a hexane extract of clove resulted in concentrations higher than 0.12% in petrolatum in local reactions. In a concentration of 1% in petrolatum in two of four patients at a moderate reaction was observed, the other two an intense reaction (large, infiltrated, dark spots with numerous vesicles) occurred (Bouhlal et al 1989 cited in Blaschek et al 2008).

In an epicutaneous test with clove powder (on filter paper moistened with water) out of 78 patients with allergy against Peru balsam reacted 36 positive. In a control group of 156 probands lacking Peru balsam allergy nobody responded positively (Niinimäki 1984).

A 22 years old patient with eczema on the hands reacted to a p. o. stress test with 2 times 100 mg clove powder in gelatine capsules with blisters on palms and fingers (Niinimäki 1984).

Clove cigarettes have been reported to cause acute respiratory problems in humans that rapidly progress to hemorrhagic pulmonary oedema or pneumonia (Gruenwald et al 2004).

In a patch test study a 10% ethanol extract of *Caryophylli flos* was investigated among other herbal preparations used in the Traditional Chinese Medicine. Out of 30 patients 8 reacted positively to clove extract (Chen et al 2003).

A root canal filling with eugenol cement resulted in a patient with a generalized urticaria. In the skin test, the patient responded positively to Peru balsam and cloves. A distributed oral provocation test with 0.1 to 0.5 mL of eugenol, in water, resulted in urticaria, which persisted for several weeks (Grade & Martens 1989).

Clove oil, 20% incorporated in petrolatum, produced in 2 of 25 healthy subjects an erythema. In concentrations of 2% and 0.2% in petrolatum no reaction were observed (Opdyke 1975 cited in Blaschek et al 2008).

Systemic effects (case report):

When trying to administer clove essential onto an aching tooth a 24 year old woman disposed accidentally on the oil on the upper lip and cheek. Although she tried to remove the essential oil a sensation of burning and inflammation occurred, which disappeared within a few hours. Subsequently a local anaesthesia and reduced sweat production in the affected areas was observed. The medical examination after 11 months revealed a dry, slightly erythematous skin with reduced pressure sensitivity. During the following 9 months the situation remained unchanged (Isaacs 1983).

Contraindications:

The medicinal use of clove essential oil should be contraindicated in cases of hypersensitivity to clove essential oil as well hypersensitivity to Peru balsam.

Serous adverse events and deaths

A 17-year-old male high-school student died of rapidly progressive inflammatory lung disease that developed hours after smoking a clove cigarette. The student was recovering from a lower respiratory infection at the time. The California Department of Health Service and Centers for Disease Control collected 110 cases of clove cigarette toxicity by 1984, two of which were fatal. Clove cigarettes have been reported to cause acute respiratory problems in humans that rapidly progress to hemorrhagic pulmonary edema or pneumonia (Gruenwald et al 2004).

During 1984 and 1985, the Centers for Disease Control received 11 case reports of clove cigarette smoking-associated acute respiratory system injury in adolescents and young adults; two deaths were also reported. The typical clove cigarette smoker inhales approximately 7 mg of eugenol per clove cigarette. The reported respiratory adverse effects included haemoptysis, bronchospasms, hemorrhagic and non-hemorrhagic pulmonary oedema, pleural effusion, respiratory insufficiency, respiratory infection, and aspiration of foreign material. The hazards of chronic long-term use of clove cigarettes are unknown because they have not been studied systematically during a long period of time (Priutt et al 1991). Clove affects cellular respiration as well. Eugenol inhibits cytochrome oxidase, thus poisoning the mitochondria (Gruenwald et al 2004).

Laboratory findings

There is no data available.

Safety in special populations and situations

Intrinsic (including elderly and children) / extrinsic factors

None known.

Drug interactions

The antiplatelet effect of clove oil may increase the risk of bleeding if taken with these medications. Clove may result in a false increase in phenytoin levels (Gruenwald et al 2004).

Assessor's comment: The proposed route of administration is the oromucosal use; the duration of use is limited. Therefore these mentioned theoretical drug interactions are not relevant for the traditional use of clove oil for the short term treatment of tooth ache or as an antiseptic mouthwash.

Use in pregnancy and lactation

No data are available. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

Overdose

Case reports:

A 7-month-old boy received 1 teaspoon of clove oil. The dose corresponds to about 500 mg / kg eugenol. On admission to hospital was an attenuation of the CNS (awake, but without a direct response to environment), leukocytosis, proteinuria, and ketonuria were observed. The also observed metabolic acidosis was attributed to the already existing diarrhoea. 3 h after ingestion, gastric lavage was performed with the addition of activated carbon. An endoscopy the next morning showed no evidence of mucosal damage in the stomach or oesophagus. After 48 h acidosis and leukocytosis were no longer detectable. The patient recovered completely and was released from hospital after 4 days (Lane et al 1991).

A 2 year old boy drank 5 to 10 mL of clove oil. After 1 hour not only mild drowsiness was observed. Within the next 3 hours, a drastic deterioration occurred with deep coma and severe acidosis. 8.5 hours after the ingestion generalized cramps occurred which were treated with diazepam. The patient had an unrecordable blood glucose level which was treated with intravenous dextrose. 24 hours after ingestion, the patient was unconscious. A severely impaired liver function and disseminated intravascular coagulopathy (therapy with plasma, heparin, antithrombin III, protein C, factor VII) was observed. The liver function deteriorated further in the following days. During the 5th day the patient awoke and on day

6 he was fully conscious. From this timepoint the symptoms gradually disappeared, the patient fully recovered (Hartnoll et al 1993).

A very similar case – ingestion of about 10 mL of clove oil by a 2-year boy resulting in convulsions, unconsciousness and severe coagulation - is described in Blaschek et al (2008).

Drug abuse

None reported.

Withdrawal and rebound

None reported.

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

None reported.

Overall conclusions on clinical safety

Clove essential oil acts in high concentrations as local irritant, allergic reactions may also be possible. However, when applied in diluted form no reports on severe adverse events are published. Clinical safety when applied correctly can be established.

3.2.6. Overall Conclusions

The positive effects of Caryophylli flos, Caryophylli floris aetheroleum and preparations thereof on inflammatory changes of the oral and pharyngeal mucosa, or in dentistry, for topical anaesthesia have long been recognised empirically. The use is made plausible by pharmacological data. There is al lack of controlled clinical studies, using herbal preparations, containing the herbal substance Caryophylli flos, Caryophylli floris aetheroleum.

In conclusion, Caryopyhlli flos, Caryophylli floris aetheroleum and its preparations can be regarded as traditional herbal medicinal products.

The potential genotoxicity of eugenol, the major constituent of the essential oil, has to be further assessed. The structural similarity to other known genotoxic carcinogens is the reason for these safety concerns.

Therefore a final benefit-risk assessment is not possible based on the current data. Experts in toxicology have to be involved in the further development of the Community monograph on Caryophylli aetheroleum.

4. DISCUSSION

The medicinal use of **Tormentillae rhizoma** can be traced in literature back to the 15th century. In fact tormentil is an old medicinal herb with a long history of traditional use as herbal tea and in liquid and dry extracts. Therefore for Tormentillae rhizoma a period of medicinal use of at least 30 years (15 years in and 15 years outside the EU) as requested by Directive 2001/83, article 16c 1 (c) or 2004/24 EC for qualification as a traditional herbal medicinal product is easily fulfilled. Its traditional use for acute unspecified diarrhoea and inflammations in the mouth or throat is plausible due to the content on tannins and the known pharmacological properties of tannins.

In some old handbooks further traditional indications were mentioned like haemostasis, prosthetic pressure points, frostbites, burnings, haemorrhoids and poorly healing wounds.

The literature search and the evaluation of the scientific information for the establishment of an assessment report and a draft monograph for the European Medicines Agency EMA were designed under several aspects.

Every indication has to be connected with a well defined herbal preparation and a well defined posology. On account of this scientific literature data bases were sought for clinical data. Formerly and up-to-date pharmaceutical literature was evaluated particularly with regard to traditional indications and preparations. Supplemental data on authorized products were collected within the EU (regulatory status overview).

In the draft monograph five herbal preparations are specified which were used traditionally: The comminuted herbal substance, two different tinctures, a liquid and a dry extract are described.

Tormentil is in medicinal use in products as single active ingredient, in combination products as well as in food supplements within the member states like shown in the regulatory status overview.

The toxicological data for Tormentillae rhizoma is limited, but with respect to the existing data, there are no safety concerns for the use as traditional herbal medicinal product. The use in children and adolescents under 18 years of age is documented. Therefore the use should be restricted to adults. Safety during pregnancy and lactation has not been established.

The available non-clinical data on tannins allowed the elaboration of an extensive overview on pharmacological topics. Antiviral, astringent, antimicrobial, anti-inflammatory, immunostimulatory, interferon inducing, molluscicide, hypoglycaemic, antioxidant, and antitumor effects were shown in different studies. There was no information found concerning clinical pharmacodynamics and only one clinical study relating to pharmacokinetic data is published. The efficacy is not confirmed by clinical data. Consequently the criteria for well-established use are not fulfilled.

Since the benefit-risk evaluation is clearly positive the traditional use of herbal preparations of Tormentillae rhizoma can be recommended in a community monograph. The medicinal use of Caryophylli flos and Caryophylli aetheroleum can be traced in literature back to the 13th century. In fact Caryophylli flos and its essential oil have been in therapeutic use for many decades. Unfortunately no medicinal products containing Caryophylli flos as the only

active ingredient could be identified in the EU member states. Therefore no monograph on clove can be established. The essential oil is in medicinal use in several products in the United Kingdom for decades. Therefore concerning *Caryophylli aetheroleum* a period of at least 30 years in medicinal use as requested by Directive 2004/24 EC for qualification as a traditional herbal medicinal product is fulfilled.

Caryophylli flos is traditionally used primarily as a spice. Traditional literature mentions the use in indications like inflammatory changes of the oral and pharyngeal mucosa, in dentistry for local anaesthesia, for dyspeptic complaints, flatulence and diarrhoea.

Caryophylli floris aetheroleum is traditionally used for external or local applications for the treatment of toothache, and minor infections of the mouth and skin, dressing of minor wounds, sore throats and coughs associated with common cold. *Caryophylli floris aetheroleum* or eugenol alone is mixed with zinc oxide as temporary filling material in dentistry. Some authors mention also myalgia, rheumatic complaints, insect bites, flatulent colic, and nausea as traditional indications. The high content of eugenol makes the medicinal use in the proposed indication plausible.

Since the efficacy of traditional herbal medicinal products is only plausible the safety must be guaranteed. In the case of *Caryophylli aetheroleum* the main component eugenol gives reason for safety concerns. Natural compounds with a similar chemical structure like safrole and methyleugenol are known as genotoxic carcinogens. Available data regarding genotoxicity of eugenol are inconsistent and equivocal. Therefore a comprehensive assessment of the risk associated with the use of *Caryophylli aetheroleum* in traditional herbal medicinal products has to be performed. In the worst case, only an assessment report will be published.

5. ABSTRACT

The goal of my diploma thesis was the development of first drafts of EU-community monographs and assessment reports *Potentilla erecta* L., rhizoma and *Syzygium aromaticum* L., flos and aetheroleum. In these documents the scientific knowledge as well as the concrete use in the EU member states should be compiled according to 'well-established use' and 'traditional use' as defined by the EU legislation. The documents were prepared in accordance with the guidelines and templates of the European Medicines Agency (EMA).

Literature search took place from August 2009 to January 2010. Handbooks of pharmacy and phytotherapy were screened in the library of Pharmacy and Nutritional Sciences and the main library of the University of Vienna as well as in the Library of the Medical University of Vienna. Articles from journals were searched in several internet databases, above all PubMed, relevant articles were obtained in full text.

Additionally data on authorised products containing tormentil or clove were collected. The assessment of the literature comprises topics such as constituents, pharmacology, pharmacokinetics, preclinical safety data, data on clinical efficacy, side effects, and interactions.

There are no sufficient clinical data published, therefore tormentil and clove essential oil are restricted to traditional use only.

There is long-documented traditional use of tormentil. Several herbal preparations exist in this context. The comminuted drug, two different tinctures prepared with ethanol-water mixtures, a liquid and a dry extract are listed in the monograph. The proposed preparations are indicated for mild diarrhoea and inflammation in the mouth and throat. The monograph, assessment report and list of references as presented were adopted after two rounds of discussions by the Committee on Herbal Medicinal Products of the EMA for public consultation.

No data could be retrieved on medicinal products containing *Caryophylli flos* authorised in the EU. Therefore no monograph for this herbal substance will be proposed. The essential however is in medicinal use since at least 30 years; the indications 'symptomatic treatment of toothache' and 'minor infections in the mouth' are plausible due to the published pharmacological properties. Before drafting a community monograph the potential genotoxicity of eugenol, the major constituent of the essential oil, has to be assessed. The structural similarity to other known genotoxic carcinogens is the reason for these safety concerns. The documents as presented in the diploma thesis are due to this ongoing discussion on safety still in the stage before the first discussion at the Committee on Herbal Medicinal Products of the EMA.

The documents developed in this diploma thesis provide the basis for a further harmonisation within the European Union regarding traditional herbal medicinal products containing tormentil or clove essential oil.

5.1. ZUSAMMENFASSUNG

Das Ziel meiner Diplomarbeit war die Entwicklung erster Entwürfe von EU-Gemeinschaftsmonographien und Abschlussberichten von *Potentilla erecta* L., *rhizoma* und *Syzygium aromaticum* L., *flos* und *aetheroleum*. In diesen Dokumenten soll das wissenschaftliche Wissen wie auch die konkrete Verwendung in den EU Mitgliedsstaaten unter Berücksichtigung des ‘well-established use‘ und ‘traditional use‘, wie es in der EU Gesetzgebung definiert wird, zusammengetragen werden. Die Dokumente wurden nach den Richtlinien und Vorlagen der EMA (European Medicines Agency) erstellt.

Meine Literaturrecherche erstreckte sich von August 2009 bis Jänner 2010. Handbücher der Pharmazie und Phytotherapie wurden in der Bibliothek für Pharmazie und Ernährungswissenschaften und der Hauptbibliothek der Universität Wien, wie auch in der Bibliothek der Medizinischen Universität Wien durchgesehen. Zeitschriftenartikel wurden in einigen Internetdatenbanken gesucht, allen voran Pubmed, relevante Artikel wurden in Volltext erworben.

Zusätzlich wurden Daten von autorisierten Produkten, welche Blutwurz oder ätherisches Nelkenöl enthalten, gesammelt. Die Beurteilung der Literatur umfasst Themen wie Inhaltsstoffe, Pharmakologie, Pharmakokinetik, Daten der präklinischen Sicherheit, Daten der klinischen Wirksamkeit, Nebenwirkungen und Interaktionen.

Veröffentlichte klinische Daten sind nicht ausreichend, deshalb sind Blutwurz und ätherisches Nelkenöl auf die traditionelle Anwendung beschränkt.

Für Blutwurz gibt es eine lang dokumentierte traditionelle Anwendung. Hier gibt es einige pflanzliche Zubereitungen. Die zerkleinerte Droge, zwei Tinkturen mit Ethanol-Wasser Mischungen, einen Flüssig- und einen Trockenextrakt findet man in der Monographie aufgelistet. Die vorgeschlagenen Zubereitungen sind bei mildem Durchfall und Entzündungen im Mund- und Rachenraum indiziert. Die präsentierte Monographie, der Abschlussbericht und die Liste der Referenzen wurden nach zwei Diskussionsrunden des ‘Committee on Herbal Medicinal Products‘ der EMA für die öffentliche Konsultation aufgenommen.

Es konnten keine Daten in Bezug auf medizinische Produkte, welche autorisiertes Caryophylli *flos* enthalten, erfasst werden. Deshalb wird für diese Pflanze vermutlich keine Monographie vorgeschlagen werden. Jedoch ist das ätherische Öl seit mehr als 30 Jahren in medizinischem Gebrauch; die Indikationen ‘symptomatische Behandlung von Zahnschmerzen‘ und ‘geringfügige Infektionen der Mundhöhle‘ sind plausibel aufgrund der publizierten pharmakologischen Eigenschaften.

Vor dem Entwerfen einer gemeinschaftlichen Monographie muss die potentielle Genotoxizität von Eugenol, dem Hauptbestandteil des ätherischen Öles, abgeschätzt werden. Die strukturelle Ähnlichkeit zu anderen bekannten genotoxischen Karzinogenen ist der Grund für die Sicherheitsbedenken. Die in der Diplomarbeit präsentierten Dokumente sind aufgrund der laufenden Diskussion hinsichtlich der Sicherheit noch einen Schritt von der ersten Diskussion des ‘Committee on Herbal Medicinal Products‘ der EMA entfernt.

Die in dieser Diplomarbeit entwickelten Dokumente liefern die Basis für eine weitere Harmonisierung innerhalb der Europäischen Union betreffend traditionelle pflanzliche medizinische Produkte, welche Blutwurz oder ätherisches Nelkenöl enthalten.

6. REFERENCES

6.1. *POTENTILLA ERECTA* (L.) RAEUSCH., RHIZOMA

COMMITTEE ON HERBAL MEDICINAL PRODUCT (HMPC)

Draft

LIST OF REFERENCES SUPPORTING THE ASSESSMENT REPORT ON:

Tormentillae rhizoma
Potentilla erecta (L.) Raeusch., rhizoma
(Tormentil)

The EMEA acknowledges that copies of the underlying works used to produce this monograph were provided for research only with exclusion of any commercial purpose.

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6.2. SYZYGIUM AROMATICUM (L.) MERRILL. ET L.M. PERRY., FLOS, AETHEROLEUM

COMMITTEE ON HERBAL MEDICINAL PRODUCT (HMPC)

FINAL

LIST OF REFERENCES SUPPORTING THE ASSESSMENT REPORT ON:

Caryophylli flos, Caryophylli aetheroleum
Syzygium aromaticum (L.) Merrill. et L.M. Perry., flos, aetheroleum
(Clove buds, Clove oil)

The EMEA acknowledges that copies of the underlying works used to produce this monograph were provided for research only with exclusion of any commercial purpose.

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