EUROPEAN MEDICINES AGENCY
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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Thymus vulgaris* L., *vulgaris zygis* L., herba

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

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Thymus vulgaris* L., *Thymus zygis* L., herba
Herbal preparation(s)	a) Liquid extract (DER 1:1), extraction solvent ethanol 24% (v/v) b) Liquid extract (DER 1:1.16), extraction solvent glycerol 85% (m/m): ethanol 25% (m/m) (0.1:2) c) Liquid extract (DER 1:2-2.5), extraction solvent ammonia solution 10% (m/m) : glycerol 85% (m/m) : ethanol 90% (v/v) : water (1:20:70:109) d) Tincture (1:10), extraction solvent ethanol 70% (v/v) e) Tincture (1:5), extraction solvent ethanol 70% (v/v) f) Soft extract (DER 5-8:1), extraction solvent ethanol 25% - 30% (v/v) g) Liquid extract fresh herb (DER 1:1.5-2.4), extraction solvent water h) Dry extract (DER 6-10:1), extraction solvent ethanol 70% (v/v) i) Dry extract (DER 1.6-2.4:1), extraction solvent ethanol 96% (v/v) j) Liquid extract (DER 1:4.5), extraction solvent ammonia solution 10% (m/m) : glycerol 85% (m/m) . ethanol 96% (v/v) : water (1.2:25:112:113) k) Dry extract (DER 7-13:1), extraction solvent water l) Comminuted herbal substance
Pharmaceutical forms	Liquid and solid dosage forms for oral use
Rapporteur	R. Länger
Assessor(s)	R. Länger

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7051
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

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1. Introduction

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

- Herbal substance(s)

Thyme, Thymi herba (European Pharmacopoeia)

Whole leaves and flowers separated from the previously dried stems of *Thymus vulgaris* L. or *Thymus zygis* L. or a mixture of both species. Minimum content of essential oil: 1.2% with minimum 40% thymol + carvacrol.

Constituents (Czygan *et al*., 2004, Hänsel *et al.,* 1994)

Essential oil: there are at least 6 chemotypes of *Thymus vulgaris* (Thompson 2003) with different compositions of the essential oil; only the 'thymol'-type with thymol as predominant compound complies with the definition in the European Pharmacopoeia. The dried herbal substance contains up to 2.5% essential oil; the main components are thymol, carvacrol, p-cymene, γ-terpinene, linalool, β-myrcene, terpinen-4-ol. Some compounds occur partly as glycosides (e.g. p-cymene-9-ol (Takeuchi *et al.* 2004), Kitajima *et al.* 2004). No differences in the composition of the essential oil were found between *Thymus. zygis* subsp. *gracilis* and subsp. *sylvestris* (Pérez-Sánchez *et al,* 2008).

Flavonoids: flavones (e.g. apigenin, luteolin, 6-hydroxyluteolin) and their glycosides, methylated flavones (e.g. cirsilineol, eriodictyol, thymonin). The content of luteolin was found to be 1.12 mg/g in the herbal substance, 3.9 mg/g in a dry extract (not specified) and 0.466 mg/g in the liquid extract prepared according to the German Pharmacopoeia (herbal preparation C) (Bazylko & Strzelecka, 2007).

Caffeic acid, rosmarinic acid: Total phenolics (calculated as caffeic acid) are best extracted with 60% ethanol (approximately 50 mg / gram plant dry matter), 30% ethanol yields app. 40 mg/g, while extraction with ethanol 96% resulted in 24 mg/g (Chizzola *et al.,* 2008). These findings are in agreement with the results published (Damianova *et al.,* 2008). However, the investigations reveal that the temperature during extraction has considerably more influence on the amount of extracted 'tannins' (only an unselective analytical method was used). An increase in temperature from 20° to 60° (ethanol 50% and 70%) resulted approximately in a triplication of the extracted amount, while increase of the duration of the extraction had only a minor influence.

Carbohydrates: up to 8% polysaccharides, approximately 1% free monosaccharides

Triterpenes: derivatives of ursolic and oleanolic acid

- Herbal preparation(s)

See section 2.1 Information on period of medicinal use in the Community.

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

This assessment refers to the use of Thymi herba as single active substance only.

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

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☒ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☒ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Latvia	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

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

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

1.3. Search and assessment methodology

First assessment 2007: Search in PubMed, search terms '*Thymus vulgaris*', '*Thymus zygis*', Thymol. Handbooks of Pharmacy and Phytotherapy, manual search in the specialised library of the University of Vienna.

For revision of the monograph 2013: Search in database '*Scopus*', search terms '*Thymus vulgaris*', '*Thymus zygis*', Thymol. Selection of articles published between 2007 and March 2013.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

2.1.1. Medicinal products

Austria

	Herbal preparation	Since
1	Comminuted herbal substance	At least since 1977
2	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	2012
3	Dry extract (DER 7-13:1), extraction solvent water	2011

Belgium

	Herbal preparation	Since
1	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	2006

Bulgaria

	Herbal preparation	Since
1	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	1997
2	Dry extract (DER 7-13:1), extraction solvent water	2012

Germany

	Herbal preparation	Since
1	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	1978
2	Liquid extract from fresh herb (DER 1:1.5-2.4), extraction solvent water This extract is often referred as expressed juice. However, as the fresh plant material is mixed with hot water and after 15 minutes contact time pressed, the term 'liquid extract' seems more appropriate compared to 'expressed juice'.	1978
3	Dry extract (DER 6-10:1), extraction solvent ethanol 70% v/v	1993
4	Liquid extract (DER 1:1.2-2), extraction solvent ammonia solution 10% (m/m) : glycerol 85% (m/m) . ethanol 96% (v/v) : water (1:20:50:130)	1994
5	Dry extract (DER 1.6-2.4:1), extraction solvent ethanol 96% (v/v)	1983

6	Liquid extract (DER 1:4.5), extraction solvent ammonia solution 10% (m/m) : glycerol 85% (m/m) : ethanol 96% (v/v) : water (1,2:25:112:113)	1978
7	Soft extract (DER 1.7-2.5:1), extraction solvent ammonia solution 10% (m/m) : glycerol 85% (m/m) . ethanol 96% (v/v) : water (1:20:70:109)	1989
8	Soft extract (DER 5.5-6.4:1), extraction solvent ethanol 30% (v/v)	1993
9	Soft extract (DER 5-8:1), extraction solvent ethanol 25% (v/v)	At least since 1976

Latvia

	Herbal preparation	Since
1	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	1996
2	Dry extract (DER 7-13:1), extraction solvent water	2012

Lithuania

	Herbal preparation	Since
1	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	2010
	Dry extract (DER 7-13:1), extraction solvent water	2011
	Liquid extract (DER 1:1), extraction solvent ethanol 30% (v/v)	2003

Poland

	Herbal preparation	Since
1	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	Before 1980

2.1.2. Information from pharmacopoeias and handbooks

	Herbal preparation	Reference	Since
1	Liquid extract (DER 1:1), extraction solvent ethanol 24% v/v	Austrian Pharmacopoeia ÖAB, current edition, monograph unchanged at least from 1960 onwards	At least since 1960
2	Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)	German Pharmacopoeia DAB, 1968 onwards	1968
3	Liquid extract (DER 1:1.16), extraction solvent glycerol 85% (m/m) + ethanol 25% v/v (0.1:2)	Czech Pharmacopoeia	1970
4	Tincture (1:5), extraction solvent ethanol 45% v/v	British Herbal Pharmacopoeia	1973
5	Fluid extract (no information on DER and extraction solvent)	German Commission E	1984
6	Tincture (1:10), extraction solvent ethanol 70% v/v	Van Hellemont, 1988; ESCOP(2003)	At least since 1973

2.1.3. Consolidated list of herbal preparations proposed for the monograph

a) Liquid extract (DER 1:1), extraction solvent ethanol 24% v/v (Austrian Pharmacopoeia)

b) Liquid extract (DER 1:1.16), extraction solvent glycerol 85% (m/m) + ethanol 25% v/v (0.1:2) (Czech Pharmacopoeia)

c) Liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts); A minimum content of phenolic compounds of 0.03% is required. The description of the manufacturing process of this extract has been improved over the years. The tradition of herbal preparations which are manufactured in accordance with the monographs from the DAB 7 onwards can be accepted.(Germany: herbal preparation 1)

d) Tincture, DER 1:10, extraction solvent ethanol 70% V/V;
1 ml corresponds to 0.1 g herbal substance. (Van Hellemont, 1988, ESCOP 2003)

e) Tincture, ratio herbal substance : extraction solvent 1:5
(British Herbal Pharmacopoeia)

f) Soft extract (5-7:1), extraction solvent ethanol 25% - 30% (v/v)
(Germany: herbal preparations 8 and 9)

g) Liquid extract fresh herb (DER 1:1.5-2.4), extraction solvent water
(Germany: herbal preparation 2)

h) Dry extract (6-10:1), extraction solvent ethanol 70% (v/v)
(Germany: herbal preparation 3)

i) Dry extract (DER 1,6-2,4:1), extraction solvent ethanol 96% (v/v)
(Germany: herbal preparation 5)

j) Liquid extract (DER 1:4.5), extraction solvent ammonia solution 10% (m/m) : glycerol 85% (m/m) : ethanol 96% (v/v) : water (1,2:25:112:113) (Germany: herbal preparation 6)

k) Dry extract (DER 7-13:1), extraction solvent water
Dry residue from the herbal tea. (Registered as THMP according to Dir 2001/83/EC as amended in several member states.)

l) Comminuted herbal substance for tea preparation.

The comminuted herbal substance is also currently available in solid dosage forms on the market as capsules containing the powder. However, none of the marketed products is documented in medicinal use since at least 30 years.

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

2.2.1. Indications reported for herbal medicinal products containing herbal preparations of Thyme

Austria Treatment of catarrhs of the upper airways; acute bronchitis; dry cough.

Belgium Indicated for cooling, sore throats and calms the cough, once the event of a serious illness excluded.

Bulgaria Mucolytic and expectorant in cough associated with cold; symptomatic treatment of acute upper respiratory tract, involving dry irritating cough and hoarseness of voice; as expectorant at cough connected with common colds.

Germany For the relief of symptoms in coughs and colds with viscous mucilage.

Latvia Bronchitis and cough with viscous sputum, and associated hoarseness; expectorant in cough associated with cold.

Lithuania Traditional herbal medicinal product used as anexpectorant in cough associated with cold.

Poland Traditional herbal medicinal product used as an expectorant for relief of cough associated with cold.

2.2.2. Indications reported for Thyme in standard literature on phytotherapy

Respiratory, thoracic and mediastinal disorders

Catarrh of the upper respiratory tract, bronchial catarrh	ESCOP 2003, Blumenthal *et al*., 1998, Fintelmann *et al*., 2002
Symptoms of bronchitis	Blumenthal *et al.,* 1998
Cough with spasms	Fintelmann *et al.,* 2002

General disorders and administration site conditions

Stomatitis	ESCOP 2003

Further traditional indications

Digestive disorders	Czygan *et al.,* 2004
Halitosis	ESCOP 2003

2.2.3. Indications proposed for the monograph

Based on the traditional medicinal use of Thyme the following indication is proposed for the monograph.

Traditional herbal medicinal product used in productive cough associated with cold. The product is a traditional herbal medicinal product for use in the specified indication exclusively based upon long-standing use.

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

2.3.1. Posology for adolescents, adults and elderly

2.3.1.1. Comminuted herbal substance for tea preparation (herbal preparation L)

	single dose	daily dose
ESCOP 2003	1-2 g, several times daily	no detailed data published
Blumenthal *et al.,*1988	1-2 g, several times daily	no detailed data published
Hänsel *et al*., 1994	1-2 g, several times daily	no detailed data published
Fintelmann *et al.,* 2002	2 teaspoons (= 2.8 g), several times daily	no detailed data published

'Several times' could be interpreted as '3-4 times daily.

2.3.1.2. Herbal preparations

	single dose	daily dose
a) Liquid extract (ÖAB)	2 g (ÖAB)	6 g
b) Liquid extract (Czech Pharm.)	no detailed data published; calculation via DER: 1.2 – 2.4 ml	no detailed data published; calculation via DER: 3.5-9.3 ml
c) Liquid extract (DAB 2006)	1.7 ml	5.1-6.8 ml
d) Tincture 1:10	40 drops	120 drops (ESCOP 2003)
e) Tincture 1:5	2-6 ml	6-18 ml (British Herbal Pharmacopoeia.)
f) Soft extract	50 mg	300 mg (according to the dosage of authorised products)
g) Expressed juice from fresh herb	10 ml	30 – 40 ml (according to the dosage of authorised products)
h) Dry extract (DER 6-10:1)	75 – 200 mg	225 – 600 mg (according to the dosage of authorised products)
i) Dry extract (DER 1.6-2.4:1)	135 mg	1-3 x daily (according to the dosage of authorised products)
j) Liquid extract (DER 1:4.5)	480-960 mg	1.44 – 3.84 g (according to the dosage of authorised products)
k) Dry extract (DER 7-13:1)	Equivalent to 1-2 g herbal substance	Equivalent to 3-8 g herbal substance

Assessor's comments

Liquid extract according to DAB 7 onwards: Traditionally, the particular liquid extract has been used in a low dosage: single dose 1-2 g, daily dose 1-4 g, which is in agreement with the monograph of the German commission E (Blumenthal et al., 1998). The scientific research on this type of extract (Gaedcke, 2004) as well as the improvement of the manufacture, which can be tracked in the editions of the German pharmacopoeia, revealed that the content of some marker compounds is less than could

be assumed from the DER of 1:2-2.5. Therefore the German national competent authority recommended a higher dosage than the traditional one. The proposed posology in this assessment report for this liquid extract has included both the traditional dosage and the higher one, which is based on the DER.

2.3.1.3. Proposed posology for adolescents, adults and elderly

	single dose	daily dose
a) Liquid extract (ÖAB)	1-2 ml	3-8 ml
b) Liquid extract (Czech Pharm.)	1.2-2.4 ml	3.5-9.3 ml
c) Liquid extract (DAB 2006)	1-4 g	1-14 g
d) Tincture 1:10	40 drops	120 drops
e) Tincture 1:5	2-6 ml	6-18 ml
f) Soft extract	50 mg	300 mg
g) Expressed juice from fresh herb	10 ml	30-40 ml
h) Dry extract (DER 6-10:1)	75-200 mg	225-600 mg
i) Dry extract (DER 1.6-2.4:1)	135 mg	135-405 mg
j) Liquid extract (DER 1:4.5)	480-960 mg	1.44-3.84 g
k) Dry extract (DER 7-13:1)	100-200 mg	300-800 mg
l) Comminuted herbal substance for tea preparation	1-2 g	3-8 g

2.3.2. Posology for children

2.3.2.1. Comminuted herbal substance

Literature references

	single dose	daily dose
ESCOP 2003	children up to 1 year: 0.5-1 g children >1-adolescent: 1-2 g	no detailed data published
Dorsch *et al*., (2002)	children up to 1 year: 0.5-1 g children >1-adolescent: 1-2 g	no detailed data published

2.3.2.2. Herbal preparations

Literature references

	single dose	daily dose
A) Liquid extract (ÖAB)	From 4 years: 1-2 g to be calculated as the herbal substance	1-6 g (Czygan *et al.,* 2004) to be calculated as the herbal substance (ESCOP 2003)
All other herbal preparations	no detailed data published	no detailed data published

Observational studies in children with Thymi herba (reported from the German national competent authority)

Studies with the liquid extract type c) according to the German Pharmacopoeia

Dethlefsen (1997, not published, data obtained from German national competent authority):
Study medication: Preparation containing 16.95% Thyme liquid extract (DAB), 1 ml corresponds to 75 mg herbal substance.
Indication: acute upper respiratory tract infection, symptoms of a bronchitis or pertussis
Average duration of treatment: 11.1 days

age	number of children	posology	single dose liquid extract	daily dose liquid extract
< 2 years	18	3-4 x 1.25 ml	0.21 ml	0.63-0.84 ml
2-6 years	105	3-4 x 2.5 ml	0.42 ml	1.27-1.68 ml
7-10 years	19	3-4 x 5 ml	0.85 ml	2.54-3.39 ml

Schlaps (1997, not published, data obtained from German national competent authority):
Study medication: Preparation containing 49.67% Thyme liquid extract (DAB); 1 ml corresponds to 0.22 g herbal substance.
Indication: acute upper respiratory tract infection, symptoms of bronchitis
Average duration of treatment: 9.57 days

age	number of children	posology	single dose liquid extract	daily dose liquid extract
< 2 years	48	3 x 1.2 ml	0.6 ml	1.8 ml
2-4 years	121	3 x 1.5 ml	0.75 ml	2.25 ml
4-6 years	123	3 x 1.7-2.0 ml	0.84-1.0 ml	2.53-3.0 ml
6-8 years	68	3 x 2.0 ml	1.0 ml	3.0 ml
8-12 years	77	3 x 2.0-2.3 ml	1.0-1.14 ml	3.0-3.4 ml
12-16 years	9	3 x 2.7-3.0 ml	1.3-1.5 ml	4.0-4.5 ml

Graubaum (2004, not published, data obtained from German national competent authority):
Study medication: Preparation containing 9% Thyme liquid extract (DAB 2006), 1 ml corresponds to 50 mg herbal substance.
Indication: acute upper respiratory tract infection, symptoms of bronchitis
Average duration of treatment: 8.8 days

age	number of children	posology	single dose liquid extract	daily dose liquid extract
1-5 years	171	2-3 x 10 ml	0.9 ml	1.8-2.7 ml
6-11 years	80	3 x 15ml	1.35 ml	4.05 ml

Kaas (2003):
Study medication: Preparation containing 8% Thyme liquid extract (DAB), 1 ml corresponds to 40 mg herbal substance.
Indication: acute upper respiratory tract infection, symptoms of bronchitis
Maximum duration of treatment: 14 days.
100 children in the age between 1 and 16 years.

age	posology	single dose liquid extract	daily dose liquid extract
<6 years	2.5 ml every 3 hours	0.2 ml	0.6-1.2 ml
6-12 years	5 ml every 3 hours	0.4 ml	1.2-2.4 ml
>12 years	10 ml 3-6 times daily	0.8 ml	2.4-4.8 ml

Dentinox (Integrierter Abschlussbericht vom 4.12.1997 für Dentinox Gesellschaft KG, Berlin, cited in ESCOP 2003)

In an open, multicentre study, 154 children aged 2 months to 14 years (mean 4.4 years) with bronchial catarrh or bronchitis were treated daily with 15 – 30 ml of Thyme syrup, containing 97.6 mg of Thyme liquid extract (DAB) per ml, for a period of 7-14 days (mean 7.9 days); 46 patients did not receive any co-medication. Compared to the start of the treatment an improvement of coughing was reported in 93.5% of patients.
Study medication: Syrup containing 10% Thyme liquid extract (DAB), 1 ml corresponds to 220 mg herbal substance.
Indication: bronchial catarrh, symptoms of bronchitis
Average duration of treatment: 7.9 days

age	number of children	posology	single dose herbal substance	daily dose herbal substance
2 months – 14 years	154	15-30 ml daily		1.5-3.0 ml

Assessor's comment

This herbal medicinal product contains the liquid extract according DAB. Therefore it may be concluded that the DER mentioned in the ESCOP monograph should be corrected to 1:2-2.5.

Study with the liquid extract from fresh herb expressed juice type g)

PädiSolvan (2001**,** not published, data obtained from German national competent authority)

Study medication: Preparation containing 75% expressed juice from fresh herb, 1 ml corresponds to 1.1 g herbal substance.
Indication: acute upper respiratory tract infection, symptoms of bronchitis
Duration of treatment: 9-12 days

age	number of children	posology	single dose liquid extract	daily dose liquid extract
< 1 years	32	2 x 5 ml	3.75 ml	7 ml
1-4 years	34	2-3 x 5 ml	3.75 ml	7-10 ml
4-10 years	37	2 x 10 ml	7 ml	14 ml
10-12 years	28	3 x 10 ml	7 ml	21 ml

Studies with combination products

There are several studies published on combinations of Thyme with other expectorants in children (Ismail *et al.,* 2003; Nauert *et al.,* 2006; Fasse *et al.,* 2006; Schmidt, 2008).

Assessor's comments on posology in children

Although numerous clinical trials provide evidence on the safe use of the liquid extract (DAB) and the expressed juice, the treatment of cough in children below 4 years of age needs supervision by a doctor. Furthermore, since traditional herbal medicinal products are intended to be used without medical supervision the oral use of Thyme preparations should be restricted to children over 4 years of age.

2.3.2.3. Proposed posology for children

Data from observational trials refer only to the liquid extract type c (DAB 2006) and to the liquid extract (= 'expressed juice', herbal preparation type g). The lower limit of age should be set to 4 years.

Children 4-12 years of age:

c) Liquid extract: Single dose: 0.5-0.9 ml
Daily dose: 2.5-4 ml, divided into 3-5 single doses.

g) Liquid extract from fresh herb: Single dose: 7-10 ml, 2-3 times daily

As a general precaution ethanol-free preparations should be preferred for children.

2.3.3. Evidence regarding the route of administration

The oral administration is the only route of administration for Thyme herb preparations in the traditional indications discussed in this assessment report.

2.3.4. Evidence regarding the duration of use

No restriction on the duration of use has been reported for Thymi herba and herbal preparations. The duration of use in clinical studies and observational studies was up to 14 days. However, in these studies the use was under medical supervision. Without medical supervision the duration of use should be restricted to 1 week.

3. Non-Clinical Data

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

3.1.1. Primary Pharmacodynamics

3.1.1.1. Spasmolytic activity

In-vitro data

Extracts

A soft extract (DER 1.7-2.5:1, extraction solvent ammonia solution 10% + glycerol 85% + ethanol 90% + water 1:20:70:109) was examined with regard to the endothelin-induced contraction in the rat

trachea (Engelbertz *et al.,* 2008). Thyme extract reduced dose-dependently in the range 2-10 mg/ml the response to endothelin. Bosentan, an endothelin receptor antagonist acted as a competitive inhibitor.

An ethanolic extract (DER 1:2 according to the Belgian Pharmacopoeia V, extraction solvent ethanol 25%, no indication whether v/v or m/m), exhibited in concentrations of 0.133 and 0.266 ml/20 ml spasmolytic activity in the guinea-pig ileum and trachea in a dose dependent manner (Van den Broucke *et al*., 1980, 1981, 1982). The Thyme extracts were found to be a non-competitive antagonist to specific agonists (acetylcholine, histamine, noradrenaline) and unspecific agonists (barium chloride). Inhibition of calcium-induced contractions on potassium–depolarised smooth muscles suggests inhibition of availability of calcium for muscle contraction. An ethanolic extract (ethanol 70%, DER not mentioned, content of thymol 0.072%, carvacrol 0.005%) of Thymi herba exhibited in concentrations between 0.2 and 2.0% v/v in the organ bath dose dependently, a antispasmodic activity on the isolated guinea-pig trachea (spasmogens: $BaCl_2$, carbachol, histamine, PGF_{2a}) (Meister *et al.,* 1999).

A soft extract (DER 1.7-2.5:1, extraction solvent ammonia solution 10% (m/m): glycerol 85% (m/m): ethanol 90% (v/v): water (1:20:70:109)) was tested on the effect on B2-receptors and mucociliary clearance (Wienkötter *et al*., 2007). The authors conclude that there is evidence for an influence of the Thyme extract on B2-receptors by both binding studies and biological effects.

Aqueous extracts of *Thymus vulgaris* (macerate and extract of 50 g herbal substance in 300 ml water, final concentration 10% herbal substance) were also tested in pre-contracted tracheal chains of the guinea-pig. The Thyme extracts showed dose dependently (0.25-1% in the test solution) relaxant effects which were similar to those of theophylline (in concentrations from 0.25-1 mM) (Boskabady *et al*., 2006).

A methanolic extract (no data on the DER and strength of the extraction solvent) was tested for its spasmolytic properties on the isolated guinea-pig ileum (Babaei *et al*., 2008). The extract induced dose-dependently a depression of contractions evoked by field stimulation. The EC_{50} was calculated with 1.7 mg/ml. The effect could be inhibited by addition of granisetrone (a 5-HT3-antagonist).

Begrow *et al.,* 2010 compared liquid Thyme extracts prepared according to the German pharmacopoeia (liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts)) and dry extracts either obtained from the DAB extract or by extraction with hot water. The liquid extract contained 0.038% thymol, a spissum extract obtained from this liquid extract contained 0.15% thymol, while in both dry extracts the thymol content was below the detection limit (<0.005%). The concentration of the Thyme extracts was fixed to 100 µg/ml. Extracts with very low thymol contents were effective in all models used except acetylcholine-induced rat ileum contraction. When Thyme extracts with thymol content according to the German Pharmacopoeia or with very low thymol content were compared, the extract with normal thymol content was more effective, both as a relaxant (rat ileum) and as an antispasmodic compound (rat trachea contraction induced by either acetylcholine, Ba^{++} or K^{+}) and in ciliary transport experiments. Thyme extracts with very low thymol content (practically free of volatile oil) were equally effective with respect to endothelin effects. When an extract with very low thymol content had been spiked with increasing concentrations of thymol, a concentration-dependent increase concerning the antispasmodic effect (Ba^{++}-induced trachea contraction) was observed. Engelbertz *et al*., 2012 investigated the antispasmodic effects of thymol-deprived extracts. A liquid extract according to the German Pharmacopoeia was prepared (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% v/v (70 parts), purified water (109 parts), after removal of a part of the extraction solvent a spissum extract remained. This extract contained 96 µg/g thymol and 41 mg/g flavonoids with luteolin-7-O-glycoside as major compound. A bioassay-guided

fractionation revealed that mainly luteolin contributes to the antispasmofic effects (-12% at 2 mg/ml; -37.1% at 10 mg/ml related to the precontracted trachea), while rosmarinic acid and apigenin do not.

Essential oil

Thyme oil shows a spasmolytic effect on the smooth muscle and a contracture involving a direct action on skeletal muscle by an unknown mechanism. The essential oils were diluted in methanol to give a final bath concentration of $5x10^{-5}$ and $2x10^{-4}$ g/ml for rat diaphragm *in-vitro* with muscle stimulated directly or via phrenic nerve and $4x10^{-6}$-$8x10^{-5}$ g/ml for field-stimulated guinea-pig ileum studies (Lis-Balchin & Hart, 1997).

Isolated substances

Isolated thymol was examined with regard to the endothelin-induced contraction in the rat trachea (Engelbertz *et al.,* 2008). Thymol was not effective. Bosentan, an endothelin receptor antagonist acted as a competitive inhibitor.

Van den Broucke *et al.,* 1980, 1982, 1983 focussed in several publications on flavonoids. Isolated flavonoids (thymonin, cirsilineol, luteolin and 8-methoxy-cirsilineol) exhibited in concentrations between 0.3 and 100 x 10^{-6} M spasmolytic activity in the guinea-pig ileum and trachea in a dose dependent manner. The flavones were found to be non-competitive antagonists to specific agonists (acetylcholine, histamine, noradrenaline) and unspecific agonists (barium chloride). Inhibition of calcium-induced contractions on potassium–depolarised smooth muscles suggests inhibition of availability of calcium for muscle contraction. Flavones induce relaxation of the carbachol contracted tracheal strip without stimulation of the beta–adrenoceptors. The tracheal relaxing effect of the flavones (pD_2 value) was about 3 decades less than that of the beta- adrenergic agonist isoprenaline.

Begrow *et al.,* 2010 investigated isolated compounds thymol and carvacrol. They had a concentration-dependent antispasmodic effect in the rat trachea either being stimulated by acetylcholine, K^+ or Ba^{++} (concentrations of thymol 19.1–191 µg/ml, carvacrol 33-330 µg/ml). Thymol at 191 µg/ml suppresses the effect of BaCl2 in the concentration range 5-15 mM. The authors concluded that in various models of antispasmodic effect (ileum and trachea) and by measuring ciliary activity, thymol and carvacrol were active, although other not identified components of Thyme extract appear to be very important as well, since extracts with very low thymol contents are also active.

Thymol has *in-vitro* an agonistic effect on α_1-, α_2- and β-adrenoreceptors; the spasmolytic activity was detectable in concentrations >10^{-6}M. In a concentration of 10^{-4}M, thymol suppressed the spontaneous contractile activity of the non-striated muscles of the stomach of the guinea-pig. In higher concentrations thymol exhibits a spasmolytic activity in the ratio of 1:10 compared to papaverine (Beer *et al*., 2007).

In-vivo data

Begrow *et al*., 2010 compared liquid Thyme extracts prepared according to the German pharmacopoeia (liquid extract (DER 1:2-2.5) extraction solvent mixture of ammonia 10% (1 part), glycerol 85% (20 parts), ethanol 90% V/V (70 parts), purified water (109 parts)) and dry extracts either obtained from the DAB extract or by extraction with hot water. The liquid extract contained 0.038% thymol, a spissum extract obtained from this liquid extract contained 0.15% thymol, while in both dry extracts the thymol content was below the detection limit (<0.005%) and investigated also the isolated compounds thymol and carvacrol. The extracts were given intragastrally at doses of 0.4 and 4.0 ml/kg to female Wistar rats. When Thyme extracts with normal thymol contents or with very low thymol contents were compared, the extract with normal thymol contents was more effective in ciliary transport experiments.

Assessor's comment

The concentrations of extracts and isolated constituents used in these experiments are too high to be considered of relevance to the mechanism of action when used in humans.

3.1.1.2. Antibacterial, antifungal, antiviral activity

Extracts

In a screening of the activity of plant extracts against Clotrimazole-resistant *Candida albicans* the methanolic extract from *Thymus vulgaris* exhibited highest activity with a MIC of 0.62 mg/ml (Inouye *et al.,* 2001).

An aqueous extract of Thyme (40 g soaked for 1 hour in 100 ml hot water) exhibited in concentrations of 5%, 10% and 20% a dose dependent inhibition of the adhesion of *Streptococcus mutans* to human buccal epithelial cells (Hammad *et al.,* 2007).

Extracts prepared with ethanol 20% and ethanol 80% (DER 1:24) were investigated on the impact on the Herpes virus infectivity in cell cultures (Reichling *et al.,* 2008). Rosmarinic acid as one of the presumed active ingredients could not be detected in the Thyme extract prepared with ethanol 20%. Both extracts were effective in the plaque reduction assay on acyclovir sensitive and acyclovir resistant virus strains. The IC_{50} was determined with 0.34 µg/ml (extract ethanol 80%) and 0.15 µg/ml (extract ethanol 20%).

Essential oil

The essential oil is antibacterial and antifungal, when tested on Gram-positive and Gram-negative bacteria, fungi, and yeasts, e.g. *Candida albicans*. The activity is mainly attributed to thymol and carvacrol (Simeon de Bouchberg *et al.,* 1976; Janssen *et al*., 1986; Menghini *et al*., 1987; Patakova *et al*., 1974; Allegrini *et al.,* 1972; Janssen, 1989; Farag *et al.,* 1986; Lens-Lisbonne *et al.,* 1987; Vampa *et al.,* 1988; Chalchat *et al.,* 1991; Giordani, 2004; Fabio *et al*,. 2007; Sokovic *et al*., 2008; Das Neves *et al*,. 2009). Oils with higher percentage of phenolic compounds show higher inhibitory activity (Penalver *et al.,* 2005). Even the vapour of Thyme essential oil (3-12 mg/l air) is highly effective against respiratory tract pathogens (Inouye *et al*., 2001). Thyme essential oil was also found to act against Methicillin resistant *Staphyloccus aureus* (MIC 18.50 µg/ml)(Tohidpour *et al.,* 2010) and to several multidrug resistant bacterial strains isolated from patients from hospital environment (Sienkiewicz *et al*., 2012). Correlations between concentrations of thymol and MIC and minimal bactericidal concentration suggest that the formation of membrane perforations is the principal mode of action of thymol against oral bacteria (Shapiro *et al*., 1995). The components of the essential oil act in an additive way, only with *Klebsiella pneumoniae* a partial synergism was observed (Iten *et al.,* 2009). In concentrations ranging from 0.08 to 0.16 µl/ml the essential oil from *Thymus zygis* subsp. *sylvestris* exhibited strong antifungal activity and was found to be not cytotoxic in skin dendritic cells (Gonçalves *et al.,* 2010). Móricz *et al.,* 2012 identified thymol, carvacrol, (-)-linalool and a-terpineol as compounds mainly responsible for the antimicrobial effect.

Tullio *et al.* 2012 investigated the effect of Thyme essential oil (not meeting the chromatographic profile according to the European Pharmacopoeia) on intracellular *Candida albicans*. At subminimal/minimal inhibitory concentrations (MIC 0.5% v/v) this essential oil significantly enhanced the intracellular killing activity by human polymorphonuclear granulocytes against *Candida albicans*, the effect was comparable to the positive control fluconazole (MIC 8 µg/ml). In an *in-vitro* killing assay without human polymorphonuclear granulocytes a progressive growth of the yeast cells at similar concentrations of Thyme essential oil was observed.

The combination of Thyme essential oil with amphotericin B indicated antagonistic profiles when tested against *Candida albicans.* The authors conclude that combinations of essential oils and antibiotics should be used with caution (Van Vuuren *et al*., 2009).

Braga *et al.,* 2007 found that a combination of thymol and eugenol acts synergistically on the alteration of the envelope of *Candida albicans* indicating potential benefits of the combination for the inhibition of *C. albicans* colonisation.

Thyme essential oil exhibited significant dose-dependent antiviral effects against Herpes simplex virus I (Astani *et al.,* 2010). The remaining infectivity was found between 81.6% of the control for a concentration of 1 µg/ml essential oil and 1.0% of the control for a concentration of 50 µg/ml. The authors found that the entire essential oil is more effective with lower cytotoxicity compared to isolated compounds of the essential oil.

The inhibitory concentration (IC_{50}) of the essential oil against herpes simplex virus type 2 *in-vitro* was 0.007%. Plaque formation was significantly reduced by more than 90% when the virus was preincubated with Thyme oil (Koch *et al*., 2008).

Assessor's comment

Data regarding the actual content of the essential oil in the herbal preparations and the finished products as well as the concentration in relevant organs after oral use is limited; the therapeutic significance of these findings cannot therefore be estimated.

3.1.2. Secondary pharmacodynamics

In-vitro data for extracts, the essential oil and isolated substances

Many compounds from the leaves of *Thymus vulgaris* (e.g. Rosmarinic acid, eriodictyol, taxifolin, luteolin, p-cymene, thymol, carvacrol) showed radical scavenging properties (Haraguchi *et al*., 1996, Dapkevicius *et al.,* 2002). In a model using CCl_4-induced hepatotoxicity the essential oil of *Thymus zygis* (thymol-type) showed a notable activity combined with a marked scavenger activity (Jimenez *et al*., 1993). Thyme ethanolic extracts and the essential oil exhibit antioxidant activity (Chizzola *et al*., 2008). Extracts prepared with ethanol 30% and 60% showed a considerable higher antioxidant activity in the DPPH assay as well as in the Fe-reduction assay compared to an extract prepared with ethanol 90%. The antioxidant activity of the extracts correlates with the content of rosmarinic acid, while for the antioxidant activity of the essential oil mainly depend on the content of phenolic compounds like thymol and carvacrol. DPPH radical scavenging activity of Thyme tea was less compared to green tea and peppermint tea, but higher than e.g. olive leaves or sage leaves (Büyükbalci & El, 2008).

Behnia *et al*., 2008 investigated the antiamebic effect of a hexane extract (DER 29:1), an extract prepared with ethanol 90% (DER 9.5:1) and of the essential oil. Minimal inhibitory concentrations against the trophozoites of *Entamoeba histolytica* were established with 4 mg/ml, 4 mg/ml and 0.7 mg/ml respectively.

Thymol in concentrations of 2.5-20 µg/ml was shown to inhibit the experimentally induced release of neutrophil elastase. The authors concluded that thymol may have a helpful effect in the control of inflammatory processes present in many infections (Braga *et al.,* 2006).

Ocaña & Reglero, 2012 concluded that thymol dose-dependently contributes to anti-inflammatory effects. Extracts obtained with supercritical CO_2 significantly reduced production and gene expression of the proinflammatory mediators TNF-α, IL-1B and IL-6 and increased these parameters in IL-10 in human macrophages in doses between 5 and 25 µg/ml.

Thyme oil inhibits prostaglandin biosynthesis (Wagner *et al.,* 1986). Rosmarinic acid has anti-inflammatory activity due to inhibition of classical complement pathway in rats and inhibition of some human PMN functions, when tested at several dosage levels and by several application *methods in-vitro* (e.g. Englberger *et al.* 1988: 0.316-3.16 mg/kg i.m. in the rat paw oedema model; Gracza *et al.,* 1985: IC_{50} of 3.37 mM in the model of inhibition of the malondialdehyde formation in human platelets)

Rosmarinic acid in concentrations up to 200 µM shows a strong antiangiogenic potential, which might be related to its anti-oxidative activity, which further resulted in the inhibition of ROS (reactive oxygen species) - associated VEGF (vascular endothelial growth factor) expression and IL-8 release (Huang *et al*., 2006). Rosmarinic acid exhibited also antiapoptotic effects on H_2O_2 induced cell injury in concentrations of 10 and 40 µM (Gao *et al*., 2005).

Thymol is a positive allosteric modulator of the GABA(A) receptor; it enhances the GABA-induced chloride influx at concentrations lower than those exhibiting direct activity in the absence of GABA (EC_{50} = 12 µM and 135 µM respectively)(Garcia *et al.,* 2006).

The essential oil of *Thymus zygis* showed acetylcholinesterase inhibition capacity with a IC_{50} = 1.1 µg/ml (Dandlen *et al.,* 2011).

A hexane extract from *Thymus vulgaris* inhibited the mitogen stimulated proliferation of lymphocytes (Amirghofran *et al.,* 2011,). Thymol was found to be responsible for this effect. A water extract of a defatted Thyme (DER app. 22:1) showed in concentrations up to 50 µg/ml immunoinhibtory effects on mitogenic and allogenic T cell proliferation (Amirghofran *et al.,* 2012).

Carvacrol as isolated compound induces apoptosis in HepG2 cells in concentrations starting from 0.05 mmol/l) by direct activation of the mitochondrial pathway (Yin *et al*., 2012).

In-vivo data for extracts, the essential oil and isolated substances

An ethanolic extract of Thyme (no detailed information available) was applied topically 2 times daily for 6 weeks in Balb/c mice with artificial leishmaniasis (Hejazi *et al.,* 2009). The lesions improved significantly better compared to placebo.

A dry extract (extraction solvent methanol 28%) exhibited a dose dependent anti-nociceptive effect after intraperitoneal injection (100–1000 mg/kg) (Taherian *et al.,* 2009).

Rosmarinic acid exhibited inhibitory activity in three *in-vivo* models in which complement activation plays a role: Reduction of oedema induced by cobra venom factor in the rat; inhibition of passive cutaneous anaphylaxis; impairment of *in-vivo* activation by heat-killed *Corynebacterium parvum* of mouse macrophages. Rosmarinic acid did not inhibit t-butylhydroperoxide-induced paw oedema in rat, indicating selectivity for complement-dependent processes (Englberger *et al.,* 1988).

Fachini-Queiroz *et al.,* 2012 investigated the anti-inflammatory activity of Thyme essential oil, thymol and carvacrol in the models of ear oedema in mice, induced pleurisy in rats and *in-vitro* chemotaxis. High concentrations of the essential oil (up from 250 mg/kg) as well as of carvacrol (up from 100 mg/kg) could reduce the exsudate volume induced by carrageenan. 10 mg carvacrol per ear could reduce the ear oedema. However, higher concentrations as well as thymol was inactive in this model.

A diet containing Thyme (up to 2%) as well as oral thymol and carvacrol (200 mg/kg) given once a day over 7 days induced the activity of phase I and phase II enzymes in the mouse liver up to 90% (Sasaki *et al*., 2005).

Five thousand ppm Thyme essential oil in the diet of Balb/c mice caused a significant reduction in macroscopic and microscopic colitis scores in TNBS colitis as well as a decrease in induced paw oedema

and ear swelling. However, in lower concentration (1250 ppm) an increase in the ear oedema was observed (Juhás *et al.,* 2008)

A water extract (30 g Thyme macerated in 60 ml water for one day, administered to mice with 500 mg/kg b.w. for 14 days) was able to alleviate increased levels of several liver enzymes due to alcohol intake (Shati & Elsaid, 2009).

Thymol and carvacrol are potent insect repellents in concentrations of about 0.05% in topical treatment (Choi *et al.,* 2002; Park *et al.,* 2005).

Abd el Kader & Mohamed, 2012 investigated the protective effects of a hot water extract of Thyme against symptoms of liver and renal toxicity induced by paracetamol in rats. Administration of 500 mg/kg b.w. of the freeze dried extract could significantly improve altered enzyme levels as well as the histological architecture of liver and kidney.

A lyophilised decoction of Thyme was administered orally in doses of 100 mg/kg to rats with induced hypertension over a period of 8 weeks (Kensara *et al*., 2012). The blood pressure could be lowered to nearly the initial values (control 136 mmHg; untreated 186 mmHg; treated 138 mmHg). Additionally the altered renal tissue could be improved by the Thyme extract.

3.1.3. Safety pharmacology

No information available.

3.1.4. Pharmacodynamic interactions

No information available.

3.1.5. Conclusions

The results from non-clinical experimental studies on Thymi herba and preparations thereof to support the proposed indications are very limited. Most effects observed in pharmacological studies were obtained in concentrations higher than expected when the herbal preparations are used and administered in the traditional way. The reported pharmacological effects are not considered contradictory to the traditional uses.

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

Absorption, Distribution, Metabolism, Elimination

Extracts

No data available

Rosmarinic acid

After oral administration of rosmarinic acid (50 mg/kg rat) the peak concentration is reached within 0.5 hours. Rosmarinic acid is present in plasma as conjugate or in the methylated form. The main metabolites are free or glucuronide conjugates. Orally administered rosmarinic acid is also degraded to caffeic acid, ferulic acid and m-coumaric acid, the majority of these degradation products is eliminated within 8 hours after oral administration (Baba *et al.,* 2004).

Pharmakokinetic interactions

In-vitro thymol and carvacrol were found to be metabolised mainly via CYP2A6 (Dong *et al.,* 2012).

A polymethylated flavone (8-methoxycirsilineol), isolated from *Thymus saturoides*, was found to be an inhibitor of CYP1A2 and CYP3A4 *in-vitro* with IC_{50} of 2.41µM and 1.71 µM respectively (Brahmi *et al*., 2011). This compound is also reported from *Thymus vulgaris* (Blaschek *et al*., 2012).

Assessor's comment

In-vitro findings on the influence of compounds on cytochrome enzymes should be interpreted with caution. No data on the levels of 8-methoxycirsilineol found in Thymus vulgaris are published. The report raises a theoretical risk of pharmacokinetic interactions but in the absence of case reports from actual interactions the clinical relevance of these findings is considered minimal at present.

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

3.3.1. Acute toxicity

A concentrated extract (extraction solvent ethanol 95%, no data on DER) produced decreased locomotor activity and slight slowing down of respiration in mice in an acute toxicity test. Oral doses were 0.5–3.0 g extract/kg body weight corresponding to 4.3–26.0 g dried plant material and these effects were produced at all dose levels (Qureshi *et al.,* 1991).

The LD_{50} of the essential oil oral route is 2.84 g/kg body weight in rats (Von Skramlik, 1959). No information on the composition of the essential oil used is available.

3.3.2. Subchronic toxicity

An increase in liver and testes weight was observed after oral administration of a concentrated extract (extraction solvent ethanol 95%, no data on DER) of plant material to mice. A dose corresponding to 0.9 g dried plant was administered daily for three months. 30% of the male animals died while in the female and control group only 10% died (Qureshi *et al.,* 1991).

A diet containing 10% leaves of *Thymus vulgaris* was fed to Wistar rats for 6 weeks. No toxic effects could be observed (Haroun *et al.,* 2002).

The oral administration of an extract prepared with methanol 80% (no data on DER published) in doses of 100 and 200 mg/kg b.w. in Fischer strain albino rats for 14 days did not alter several kidney and liver function parameters (Oyewole *et al.,* 2010).

3.3.3. Mutagenicity

Thyme essential oil had no mutagenic or DNA-damaging activity in either the Ames test (strains TA1535, TA1537, TA98, TA100, with and without metabolic activation, 0.25 and 0.5 µl essential oil/plate) or Bacillus subtilis rec-Assay (10 µl and 30 µl essential oil)(Zani et al., 1991). Although the Ames test lacked one of the currently used strains the negative result could be regarded reliable, because the paper contains adequate description of findings and because the Ames test is supplemented by the rec-assay.

This was confirmed by Shoeibi *et al.,* 2009: Thyme essential oil in concentrations of 50–2000 µg/ml did not show signs of mutagenicity in an Ames test using *Salmonella typhymurium* strain TA100 with and without rat liver S9.

Thymol did not show mutagenicity in *Salmonella typhimurium* strains TA97, TA98 and TA100, with and without S9 metabolic activation and 20 minutes standard preincubation time (Azizan *et al.,* 1995,).

Stammati *et al.,* 1999 determined relative cytotoxicities of thymol and carvacrol and assessed their potential genotoxicity in short-term assays. Both substances inhibited the colony-forming ability of Hep-2 cells in dose-dependent manner. The results of an AMES-test in strains TA100 and TA98 were ambiguous. Both substances were marginally more toxic to repair-deficient strain than to its repair-proficient counterpart. The substances produced elevated revertant numbers in the strain TA100, but not to a level generally considered significant. Effects in the SOS chromotest are interpreted as signs of toxicity rather than real SOS induction. The authors conclude that the genotoxic potential of thymol and carvacrol is very weak.

Concentrations of thymol and γ-terpinene above 0.1 mM significantly induced DNA damage in human lymphocytes, however, below these concentration thymol and carvacrol significantly reduced the oxidative DNA damage induced by H2O2 (Aydin *et al*. 2005) or imidazolquinoline and mitomycin C (Aydin et al., 2005a).

Thymol and carvacrol at IC_{50} concentrations reduced the level of DNA-lesions caused by H_2O_2 in $HepG_2$ and colonic Caco-2 cells (Horvathova *et al*., 2006).

Thymol in concentrations up to 520 µM did not increase the frequencies of chromosome aberrations in Syrian hamster embryo cells compared to the control cells (Hikiba *et al.,* 2005).

Ündeğer *et al*, 2009 investigated the genotoxicity of thymol and carvacrol using the comet assay. V79 Chinese hamster lung fibroblast cells were treated with 1, 5 and 25 µM thymol or carvacrol. Only at 25 µM thymol caused some clastogenic DNA damage. The authors conclude that thymol and carvacrol are free of a clastogenic activity at biologically relevant concentrations.

Rosmarinic acid did not show DNA damage in rat brain tissue in concentrations up to 8 mg/kg (Pereira *et al.,* 2005).

Azirak & Rencuzogullari, 2008 investigated the *in-vivo* genotoxic effects of carvacrol and thymol in bone marrow cells of rats. Both carvacrol (10, 30, 50, and 70 mg/kg b.w. intraperitoneally) and thymol (40, 60, 80, and 100 mg/kg b.w. intraperitoneally) significantly induced the structural and total chromosome abnormalities (CA) in bone marrow cells for all treatment periods (6, 12, and 24 h) when compared with control. Both carvacrol and thymol showed similar effects with the positive control urethane on induction of the percentage of structural and total CA at the highest concentrations except the effects of carvacrol for 6 h treatment (70 mg/kg b.w. and 100 mg/kg b.w., respectively). In addition, carvacrol induced the numerical CA at all concentrations when compared to control and at two highest concentrations (50 and 70 mg/kg b.w.) when compared to solvent control. Thymol also induced the numerical CA especially at the highest concentration (100 mg/kg b.w.) for all treatment periods.

3.3.4. Reproduction toxicity, fertility

Thyme essential oil consisting of 48% p-cymene and 24% thymol (0.25% essential oil in the feed over 2 weeks and during first 4 days of pregnancy, n=15, number of embryos: 126) showed no influence on the growth and development of mouse embryos *in-vivo* (Domaracky *et al.,* 2006).

3.3.5. Additional data

An extract of Thyme prepared with methanol (concentration not stated) was tested together with other extracts and isolated plant phenols for its effect on HepG2/C3A cells and MH1C1 cells in order to predict liver toxicity (Liu *et al*., 2011). Cluster analysis of eight parameters obtained from each cell

type revealed that in HepG2/C3A cells the Thyme extract tested in a concentration of 1000 µg/ml was grouped together with the solvent control (= no toxicity), in MH1C1 cells it was grouped together with green tea extract in a cluster indicating toxic effects on the cells.

Assessor's comment:

As the toxic effects were observed only in the cell line obtained from rats and not in the human cell line the results do not indicate a risk with the medicinal use of Thyme.

3.4. Overall conclusions on non-clinical data

Results from non-clinical experimental studies on Thymi herba and preparations thereof to support the proposed indications are very limited. Most effects observed in pharmacological studies were obtained with concentrations higher than expected when the herbal preparations are used and administered in the traditional way. The reported pharmacological effects are not considered contradictory to the traditional uses.

Specific data on pharmacokinetics are not available. Non-clinical results regarding pharmacokinetic interactions are not considered relevant to the medicinal use of Thyme.

Non-clinical information on safety is limited. The oral administration of Thyme herb preparations can be regarded as safe, especially at therapeutic doses proposed due to their long-standing use.

Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed therefore the requirements for a list entry are not fulfilled.

4. Clinical Data

4.1. Clinical Pharmacology

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

No data available.

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

Thymol

After a single oral dose of Thyme dry extract (corresponding to 1.08 mg thymol) only the sulfate could be detected in the human plasma, but not the free thymol nor the glucuronide. The sulfate could be detected 20 minutes after dosing; maximum plasma levels were reached after about 2 hours. Thymol could be detected in the plasma up to 38 hours; renal elimination was completed within 24 hours. Elimination half-life was determined as 10.2 hours (Kohlert *et al.,* 2002).

4.2. Clinical Efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

4.2.2.1. Clinical trials in indications associated with cough

Studies with Thyme preparations as the sole active ingredient

In a randomised, double-blind, comparative study, 60 patients with productive cough complaints resulting from uncomplicated respiratory infections were treated with Thyme syrup (3 x 10 ml daily, n=31, no details regarding DER, extraction solvent and amount of herbal preparation in the syrup) or a bromhexine preparation (n=29) for 5 days. No significant difference was observed between Thyme syrup and bromhexine in self-reported alleviation of the complaints on days 2 and 5 of treatment (Knols *et al*., 1994).

Assessor's comment

This study was performed in a small population. Due to the small number of patients, the lack of a placebo group and o the inappropriate characterisation of the study medication, this study cannot be used to support the use of Thyme syrup for a well- established use indication.

Studies with combinations:

The results of clinical trials of combinations of Thyme with other medicinal plants can be used for the assessment of safety only.

For a detailed discussion of the clinical trials see the assessment report on the HMPC monograph on *Thymus vulgaris* L. and *Thymus zygis* L., herba and *Primula veris* L. and *Primula elatior* (L.) Hill, radix). http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2013/04/WC500140967.pdf

4.2.2.2. Clinical trials in other indications

Herbal preparations

No data available

Isolated compounds

There is evidence that thymol is active against cariogenic and periodontopathogenic bacteria (Shapiro *et al.,* 1995). A comparison of different mouthrinses (containing thymol, chlorhexidine, povidon, hydrogen peroxide) showed no differences in the papillary bleeding score and in plaque index between the treatment with thymol and water (Maruniak *et al.,* 1992).

The use of a combination of thymol, menthol, methyl salicylate and 1,8-cineol as a dentifrice over 6 months did not show statistically significant differences between the vehicle and the essential oil group. Neither development of bacterial resistance nor emergence of opportunistic pathogens could be observed (Charles *et al.*, 2000).

Many clinical trials are published which investigate the efficacy of combinations of chlorhexidine and thymol (Twetman *et al.,* 1999). The contribution of thymol to the overall efficacy cannot be estimated.

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

In an open, multicentre study (Dentinox Integrierter Abschlussbericht vom 4.12.1997 für Dentinox Gesellschaft KG, Berlin, cited in ESCOP 2003), 154 children aged 2 months to 14 years (mean 4.4 years) with bronchial catarrh or bronchitis were treated daily with 15 – 30 ml of Thyme syrup, containing 97.6 mg of Thyme liquid extract (2 – 2.5: 1) per ml, for a period of 7-14 days (mean 7.9 days); 46 patients did not receive any co-medication. Compared to the start of the treatment an improvement of coughing was reported in 93.5% of patients.

Assessor's comments

This company report is typical of an open study sponsored by the marketing authorisation holder. The extract is stated to be a liquid extract but this seems unlikely in view of the DER. It is assumed that the liquid extract according to the DAB (with a DER of 1: 2-2.5) has been tested. Nevertheless this open study on the use of Thyme syrup in young children contributes to the documentation of the safe use of Thyme syrup as traditional herbal medicinal product in general.

Koch (2003): In this observational study children were treated with soft extract obtained from the liquid extract according to the German Pharmacopoeia. In total 204 children between 1 and 12 years of age were included in this observational trial. Due to obvious contradictions within the publication no calculation of the actual posology of the soft extract can be performed. Only general statements on efficacy and safety are published.

Assessor's comments

No data on the actual numbers of children per age group are presented. This reference contributes to the evidence of the safe use of this soft extract in the paediatric population.

The findings of the observational studies mentioned in section "2.3 Evidence regarding the specified posology" support the plausibility of efficacy of preparations of Thymi herba in the indication 'cough associated with cold'. They do not fulfil the criteria necessary for placing the respective preparations in the 'well established use' category.

4.3. Overall conclusions on clinical pharmacology and efficacy

Randomised, placebo-controlled clinical trials for herbal preparations with Thyme as the only active ingredient are not published. Therefore all herbal preparations have to be assigned to 'traditional use'. The published data on pharmacology and from observational trials support the safe traditional oral use of herbal preparations of Thyme for the treatment of cough associated with cold.

5. Clinical Safety/Pharmacovigilance

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

Clinical trials with Thyme herbal preparations from Thyme as the only active ingredient

Liquid extract according to the German Pharmacopoeia, clinical trials in children

Dethlefsen (1997, not published, data obtained from German national competent authority):
No adverse events were observed.

Schlaps (1997, not published, data obtained from German national competent authority):
One patient suffered from nausea because of the bad taste, no further adverse events were observed.

Graubaum (2004, not published, data obtained from German national competent authority):
Adverse events: a 2-year-old child showed repeating vomiting about 1 1/2 hours after administration of the preparation; a 5-year-old child showed repeating vomiting. A 1-year-old child had repeated diarrhoea, another 1-year-old child showed an exanthema of the neck and neckline.

Kaas (2003):
No adverse events reported.

Dentinox (Integrierter Abschlussbericht vom 4.12.1997 für Dentinox Gesellschaft KG, Berlin, cited in ESCOP 2003)
No adverse events are published.

Study with the liquid extract from fresh herb, clinical trials in children

PädiSolvan (2001, not published, data obtained from German national competent authority):
Two adverse events were observed: a 5-year-old child had vomiting and soft faeces on the fourth day of treatment; a 4-year-old child had soft faeces from the beginning of the treatment.

Clinical trials with herbal preparations from Thyme in combination herbal medicinal products

Ernst (1997):
Herbal preparation: 60 mg dry extract of Primulae radix DER 6-7:1, solvent ethanol 47.4% v/v + 160 mg dry extract of Thymi herba DER 5.9-10:1, extraction solvent ethanol 70% v/v:
The rate of adverse events was below 1% (in 3140 adults 0.64%, in 1490 children 0.60%).

Kemmerich (2007):
Herbal preparation: Dry extract from Thyme (DER 6-10:1), extraction solvent ethanol 70% v/v and dry extract from Primula root (DER 6-7:1), extraction solvent ethanol 47.4% v/v
No difference in the frequency or severity of adverse events was observed. Severe or serious adverse events were not reported. In the verum group 1 case with Eustachian tube disorder and 1 case of back pain were labelled as moderate, 1 case of otitis externa as mild.

Gruenwald (2005):
Herbal preparation: Liquid extract from Thyme (DER 1:2-2.5), extraction solvent ammonia solution 10% m/m : glycerol 85% m/m : ethanol 90% v/v : water (1:20:70:109) and tincture from Primula root (Ratio herbal substance to extraction solvent 1:5), extraction solvent ethanol 50% v/v
No serious adverse events were observed. Five adverse events occurred in the placebo group, 2 in the verum group (stomach ache and nausea were considered to be related to the study medication).

Gruenwald (2006):
Herbal preparation: Liquid extract from Thyme (DER 1:2-2.5), extraction solvent ammonia solution 10% m/m: glycerol 85% m/m : ethanol 90% v/v : water (1:20:70:109) and liquid extract from Primula root (DER 1:2-2.5), extraction solvent ethanol 70% m/m)
No data on safety.

Ismail (2003):
Herbal preparation: Dry extract from Thyme (DER 6-10:1), extraction solvent ethanol 70% v/v or liquid extract from Thyme (DER 1:2-2.5), extraction solvent ammonia solution 10% m/m : glycerol 85% m/m : ethanol 90% v/v : water (1:20:70:109) combined with dry extract from Primula root (DER 6-7:1), extraction solvent ethanol 47.4% v/v or liquid extract from ivy leaves (DER 1:1), extraction solvent ethanol 70%
Frequency of adverse events lower compared to ambroxol and N-acetylcystein.

Kemmerich *et al.* (2006):
Herbal preparation: liquid extract from Thyme (DER 1:2-2.5), extraction solvent ammonia solution 10% m/m : glycerol 85% m/m : ethanol 90% v/v: water (1:20:70:109) combined with liquid extract from ivy leaves (DER 1:1), extraction solvent ethanol 70%
No differences in frequency of adverse events between placebo and verum group. Medication was well tolerated, no severe or serious adverse events occurred.

Büechi (2005):
Herbal preparation: syrup containing (per 5 ml) 0.07 g dry extract of Hederae folium (DER 4-8:1), 1.7 g decoction of Thymi herba and Anisi fructus (DER 1:3.5-4 in relation to Thyme), aniseed should only enhance the flavour.

No serious adverse events, one adverse event not attributable to the study medication.

5.2. Patient exposure

No data available.

5.3. Adverse events and serious adverse events and deaths

Herbal preparations

ESCOP (2003):
In very rare cases hypersensitivity reactions have been reported.

Czygan (2004):
"Thyme and preparations thereof are normally without risk and only very rarely allergens. Cross-sensitivity has been observed for plants of the Lamiaceae family, so allergic reactions cannot be ruled out."

Benito (1996):
Reported that plants belonging to the Lamiaceae family appear to show cross- sensitivity.

Hänsel (1994):
In very rare cases allergic reactions may occur due to the content of thymol.

Adverse events due to thymol

Thymol has been used orally in folk medicine as a vermifuge at therapeutic doses (0.3 – 0.6 g, max. 1 g). Thymol in these concentrations caused abdominal pain and transient collapse (Czygan *et al.,* 2004).

Assessor's comment

The symptoms described above may also be due to the worm infections. The doses of thymol correspond to 62-208 g herbal substance. These doses exceed the recommended daily doses by far. With the recommended amounts of Thyme preparations only approximately 38 mg of thymol are administered. The proposed dosage of the pure essential oil (25 drops per day) corresponds to approximately 300 mg thymol. However, no adverse reactions from the oral use of the essential oil are published.

Essential oil
In concentrations higher than 8% in Vaseline irritation of the skin may occur. The daily application in gargles, mouthwashes and toothpastes over a longer period (no exact data available) may cause allergic reactions (Hänsel *et al.,* 1994).

Thyme dust
Thyme dust, which may occur during processing of the herbal substance, can cause contact dermatitis and asthma (Lemiere *et al.,* 1996; Spiewak *et al.,* 2001).

Case reports
Germany has received 17 case reports for allergic reactions (urticaria, skin rashes, bronchospasm, asthma attack, anaphylactic shock).

Martinez-Gonzalez (2007):
After a 24-hr application of a poultice prepared from rosemary, Thyme, arnica, chamomile and horsetail a 45-year-old man developed an acute cutaneous eczematous lesion with tendency to form vesicles and blisters. A patch test revealed *Rosmarinus* and *Thymus* as responsible for this reaction.

One case of anaphylactic shock has been reported to the German National Competent Authority.

Yürüktümen (2011):
Reported a case of acute hepatitis after ingestion of 25 ml of Thyme essential oil obtained from a local market in Turkey. The patient developed nausea, vomiting and diarrhoea, and was subsequently admitted to the emergency unit, with high transaminase levels. The patient was placed in an observation unit for two days. His elevated aminotransferase levels and symptoms gradually decreased during the observation period.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Pregnancy and lactation
Safety during pregnancy and lactation has not been established. No adverse effects have been reported from the use of Thyme herb as a medicinal product during pregnancy and lactation.

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

Fertility
No data available.

Interactions
None reported.

Foster (2003):
Reported that an aqueous Thyme extract significantly inhibited several iso- forms of CYP 450 *in-vitro*.

5.6. Overall conclusions on clinical safety

Data from clinical trials support the safe use of herbal preparations of Thyme in the indication ‘cough associated with cold’. The reported adverse events are mild. Therefore a positive benefit-risk-ratio can be assumed.

6. Overall conclusions

The expectorant effects of Thyme preparations have long been recognised empirically. The use in cases of productive cough is made plausible by pharmacological data, comparative and observational clinical studies.

In conclusion, herbal preparations of Thyme can be regarded as traditional herbal medicinal products. The available clinical studies are not sufficient to qualify preparations from Thyme herb for well-established use indications.

Tests on genotoxicity have been performed with the essential oil and with isolated substances (thymol, rosmarinic acid) only; no data are available on total extracts therefore the requirements for a list entry are not fulfilled.

Annex

List of references