EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

13 September 2011
EMA/HMPC/337067/2011
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Tilia cordata* Miller, *Tilia platyphyllos* Scop., *Tilia x vulgaris* Heyne or their mixtures, flos

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

Draft

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Tilia cordata* Miller, *Tilia platyphyllos* Scop., *Tilia* x *vulgaris* Heyne, flos or their mixtures
Herbal preparation(s)	a) Comminuted herbal substance b) Liquid extract (DER 1: 1), extraction solvent ethanol 25% V/V c) Tincture (ratio of herbal substance to extraction solvent 1:5), extraction solvent ethanol 45% V/V
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use. Herbal preparations in liquid dosage forms for oral use.

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

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1. Introduction

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

- Herbal substance(s)

Lime tree (Linden) is a tall deciduous tree native throughout Europe as far north as 65^{o} in latitude, which can grow to heights approaching to 30 metre. It is found in the wild and purposely planted in gardens. It is also cultivated in Europe and North America while the material of commerce originates mainly from Balkan countries such as Bulgaria, Romania, former Yugoslavia, Turkey and in part from China.. Lime tree bark is smooth and gray and its leaves are heart-shaped. The 5-petalated, yellow white flowers are collected in full bloom, dried and preserved under low-moisture conditions (Blumenthal *et al.*1998).

Tiliae flos , common lime flower, consists of the whole dried inflorescence of *Tilia cordata* Miller, of *Tilia platyphyllos* Scop., of *Tilia x vulgaris* Heyne (fam. *Tiliaceae*), (Ph.Eur. 7.1).

These species are preferred because the tannin and mucilage content in their flowers produce more favourable teas and extracts (Blumenthal *et al.* 1998).

Tilia has faint aromatic odour and faint sweet and mucilaginous taste.

Lime flower's inflorescence is yellowish-green. The main axis of the inflorescence bears a linguiform bract, membranous, yellowish-green, practically glabrous, the central vein of which is joined for up to about half of its length with the peduncle. The inflorescence usually consists of 2 - 7 flowers, occasionally up to 16. The sepals are detouched easily from the perianth; they are up to 6 mm long their adaxial surface and their borders are strongly pubescent. The 5 spatule, thin petals are yellowish-white, up to 8 mm long. They show fine venation and their borders only are sometimes covered with isolated trichromes. The numerous statements are free and usually constitute 5 groups. The superior ovary has a pistil with a somewhat 5-lobate stigma.

Lime flower comprises of the dried inflorescence of *Tilia cordata* Miller, of *Tilia platyphyllos* Scop., of *Tilia x vulgaris* Heyne (fam. *Tiliaceae*), or a mixture of these and is used in herbal medicine. (VIth Hungarian Pharmacopoeia 1970; BHP 1976; Bradley 1992; HagerROM 2010, for Herbal Medicines 2007).

Common names: Lime flower, lime tree, European Lime, basswood, lime tree, Linden tree.

Synonym(s)

Tilia cordata Mill. = *Tilia officinarum* Crantz, *Tilia officinarum* Crantz subsp. *Officinarum* pro parte, *Tilia ulmifolia* Scop., *Tilia parvifolia* Ehrh. ex Hoffm., Small leaved Lime

Tilia platyphyllos Scop. = *Tilia officinarum* Crantz, *Tilia officinarum* Crantz subsp. *Officinarum* pro parte, Large-leaved Lime

Tilia x vulgaris Hayne, a hybrid of the above (*Tiliaceae*)= *Tilia x europaea* auct. non L.,

Chemical constituents according to existing references (BHP 1976, Bradley 1992; Barnes et al. 2007, Duke 1985, Review Nat Prod 2005)

- **Acids** - Caffeic acid, chlorogenic acid and p-coumaric acid
- **Amino acids** - Alanine, cysteine, cystine, isoleucine, leucine, phenylalanine and serine

- **Carbohydrates** - Mucilage polysaccharides (3%). Five fractions identified yielding arabinose, galactose, rhamnose, with lesser amounts of glucose, mannose, and xylose; galacturonic and glucuronic acids;(Kram and Franz 1985; Yakovlev, 1985) gum
- **Flavonoids** - Kaempferol, quercetin, myricetin and their glycosides (mainly Kaempferol-3-O-β-D-(6″-E-p-coumaroyl)-glucopyranoside – tiliroside) (Nowak 2003)
- **Volatile oil** - (0.02% to 0.1%) Many components including alkanes, phenolic alcohols and esters, and terpenes including citral, citronellal, citronellol, eugenol, limonene, nerol, a-pinene and terpineol (monoterpenes), and farnesol (sesquiterpene) (Fitsiou *et al.* 2007; Buchbauer *et al.* 1999; Rădulescu & Oprea 2008; Ahmadi & Mirza 1999; Toker *et al.* 1999)
- **Other constituents** - Saponin (unspecified), tannin (condensed) and tocopherol (phytosterol)

Microscopic tests are used for detecting common adulterations with other species (mainly *Tilia tomentosa* = *Tilia argentea*). According to European Pharmacopoeia (Ph.Eur. 7.1.), a maximum of 2% of foreign matter can be determined on 30 g. There are no inflorescences with a bract bearing at the abaxial face stellate, five to eight-rayed trichomes and floers having an apparent double corolla by trabsformation of five stemens into-petal-line staminoids and having a pistil which is not lobular nor indented. Hexamerous flowers occur only occasionally (*Tilia americana* L., *Tilia tomentosa* Moench)

The herbal substance should contain not less than 15% water-soluble extractive (BHP 1976), while the Pharmacopoeia of Hungary (VIth Hungarian Pharmacopoeia 1970) requires not less than 18% water-soluble extractive.

The ratio of tannins to mucilage appears to be important in determining the flavour of teas prepared from lime flowers. Those teas with a high (2% or greater) tannin level and low mucilage content produce the more flavourful teas, Flowers from *Tilia cordata* and *Tilia platyphyllos* contain relatively more tannin than mucilage (Blumenthal *et al.* 2000). More than 2 dozen additional minor compounds have been identified in the wood, flowers and fruits of lime. The fragrant components of the flowers degrade rapidly under conditions of high moisture (HagerROM 2010, PDR for Herbal Medicines 2007, Review Nat Prod 2005).

- Herbal preparation(s)

<Rapporteur to include text>

- 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.
- There are several combinations registered in the EU
  - herbal tea containing Plantaginis folium(25%), Althaeae radix (20%), Cynosbati fructus sine semine (20%), Liquiritiae radix (15%), Serpylli herba (15%), **Tiliae flos (5%)** (Czech Republic)
  - herbal tea containing Foeniculi dulcis fructus (15%), Sambuci nigrae flos (25%), **Tiliae flos (25%)**, Plantaginis folium (20%), Liquiritiae radix (15%) (Czech Republic)
  - herbal tea containing Tiliae flos & Salicis cortex (Germany)
  - herbal tea containing Tiliae flos, Thymi berba & Anisi fructus,Thymi herba, Foeniculi amari fructus, Lichen islandicus (Germany)

This monograph refers only to Tiliae flos.

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

Member State	Regulatory Status (products, indications)
Austria	**TU** Herbal tea, since 1994 **Posology** For oral use in adults and adolescents over 12 years 1 - 2 times daily a cup of fresh prepared infusion (2-4 g herbal substance) Indication The traditional indication is: for the relief of early symptoms of common cold
Bulgaria	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Cyprus	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Czech Republic	**TU** Herbal tea, since 2000 *Posology:* 1. for oral use Adults: 1.5 g/250 ml of boiling water 2 - 3 times daily Adolescents/children 4 - 12 years of age: 1.5 g/250 ml of boiling water 2 times daily Children 1 - years of age: 1.5 g/250 ml boiling water once daily *Indication:* Traditionally used as an adjuvant for treatment of catarrhs of upper respiratory tract and cold associated with dry, irritating cough *Contraindication:* Hypersensitivity to the herbal substance/ Special warning: If dyspnoea, fever or purulent sputum occurs, a doctor should be consulted immediately. *Other information on relevant combination products:* 1. Herbal tea containing Plantaginis folium(25%), Althaeae radix (20%), Cynosbati fructus sine semine (20%), Liquiritiae radix (15%), Serpylli herba (15%), Tiliae flos (5%) – on the market since 1995 – for oral use, adjuvant for treatment of catarrhs of upper respiratory tract associated with dry cough 2. Herbal tea containing Foeniculi dulcis fructus (15%), Sambuci nigrae flos (25%), Tiliae flos (25%), Plantaginis folium (20%), Liquiritiae radix (15%) – on the market since 1997 – for oral use – indications: for treatment of common cold associated with elevated temperature; inflammations of oral cavity and upper respiratory tract; diaphoretic effect
Denmark	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market

Estonia	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Finland	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
France	Only in combination
Germany	**WEU** 9 Herbal teas since 1976 *Posology:* for oral use in adults and adolescents over 12 years 1 - 2 times daily a cup of fresh prepared infusion (2 - 4 g herbal substance) or 1 - 2 cups very hot as "sweat infusion" before going to bed. *Indications:* Used as a diaphoretic in feverish colds 2 authorised combination products 1. herbal tea containing Tiliae flos & Salicis cortex 2. herbal tea containing Tiliae flos, Thymi herba & Anisi fructus, Thymi herba, Foeniculi amari fructus, Lichen islandicus single active ingredient: 9 herbal teas combination products: 11 herbal teas
Greece	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Hungary	**TU** Cab be found in registered products only in combinations, but it can be bought as herbal substance in pharmacies and used according to the literature Herbal tea (Augustin *et al.*1948) *Posology:* (VIth Hungarian Pharmacopoeia 1967; English version 1970) For oral use as an infusion Usual single dose 0.5-1 g Usual daily dose 2.5-5 g *Indication:* to promote sweating in the case of common cold, chronic coughing and catarrh

Lithuania	**TU** Herbal tea, since 1995 *Posology:* Children older then 2 years, adolescents and adults Single dose 1.5-2 g of comminuted herbal substance for infusion. Daily dose 4-6 g of comminuted herbal substance for infusion *Indication:* Traditional herbal medicinal product used as expectorant in dry cough associated with cold and to promote perspiration in case of fever
Netherlands	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Norway	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Poland	Herbal tea, since 1991 *Posology:* for oral use in adults and adolescents over 12 years *Indication:* Mild antipyretic and diaphoretic agent in common cold and in cough Hypersensitivity reactions (i.e. urticaria)
Portugal	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Slovakia	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Slovenia	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
Spain	Only combination products containing *Tiliae* flos are registered
Sweden	No authorised herbal medicinal products containing *Tilia* flos as a single drug preparation are on the market
United Kingdom	Only combination products containing *Tilia* flos are registered

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Herbal tea as the only active ingredient since 1994
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Multi-component herbal teas since 1962
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	One herbal tea, since 2000
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Only in combination products
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Herbal tea since 1976
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products on the market
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only in combinations
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Herbal tea since 1995
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Herbal tea since 1991
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not known
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered

Member State	Regulatory Status				Comments
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Only combination products are registered *Tilia* flos can be freely sold as food
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products registered
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combination products containing *Tilia* flos are registered

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

Search terms: *Tilia cordata* Miller, *Tilia platyphyllos Scop. Tilia* x *vulgaris* Heyne, flowers, Tiliae flos. *Tilia* sp., tiliroside,
Databases: Pubmed, Medline, HealLink, scopus.
Libraries: University of Athens, Lab. Of Pharmacognosy and Chemistry of Natural Products of the University of Athens.The regulatory status of Tiliae flos preparations in EU was requested by e-Mail in 2010.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Since the Middle Ages, the lime flowers have been used as a diaphoretic to promote perspiration. In addition, the flowers have been used traditionally as tranquiliser and to treat headaches, indigestion and diarrhoea. Infusions of the flowers make a pleasant-tasting tea. Traditionally lime flowers were added to baths to quell hysteria and steeped as a tea to relieve anxiety-related indigestion, heart palpitation and vomiting (Blumenthal *et al.* 1998; 2000). Several sources report the lore that lime flowers were once believed to be so effective in treating epilepsy that one could be cured simply by sitting beneath the tree. Sugar is obtained from the sap of the tree and the seed oil resembles olive oil. In Greek mythology, "Philyra" a nymph, was transformed into a lime tree after begging the gods not to leave her among the mortals (Barnes *et al.* 2007, Duke *et al.*1985, Review Nat Prod 2005, BHP 1976)

The lime flowers are referred in the VIth Hungarian Pharmacopoeia (1967, English version 1970) with the following posology: usual single dose: 0.5 - 1 g usual daily dose: 2.5 - 5 g. In the Hungarian book by Augustin *et al.* 1948 it is mentioned that the lime flowers are used in the form of infusion for indication of promoting sweating in the case of common cold, chronic coughing and catarrh even they are used for preparing candies to relieve cough.

They can be found also in the first edition of the Czechoslovak Pharmacopoeia in 1947, as well as in the current version of Czech Pharmacopoeia (2009, suppl. 2010).

In Germany, lime flower approved in the Commission E monographs (Blumenthal *et al.* 1998), and the tea form is official in the German Standard Licence monographs, and it was also official in the

pharmacopoeia of the former German Democratic Republic (Blumenthal *et al.* 1998). It is used as a component of common cold and antitussive preparations and also as an urological and sedative drug.

In German paediatric medicine, it is used as a diaphoretic component of an influenza tea for children comprised of lime flower, willow bark, meadow-sweet flower, chamomile flower and bitter orange peel. It is also a primary component of "Schweisstreibender Tee" (diaphoretic tea) composed by lime flower, peppermint leaf, meadowsweet flower and bitter orange. In Switzerland is known a comparable diaphoretic tea composed of lime flower, elder flower, mint leaves, and jaborandi leaf (Blumenthal *et al.* 1998).

Medicinal Use

The Commission E approved lime flower for colds and cold-related coughs (Blumenthal *et al.* 1998; 2000). Lime flower is stated to possess sedative, antispasmodic, diaphoretic, hypotensive, emollient, diuretic and mild astringent properties. Traditionally it has been used for migraine, hysteria, arteriosclerotic hypertension, feverish colds, and specifically for raised arterial pressure associated with arteriosclerosis and nervous tension (Barnes *et al.* 2007)

Bradley 1992, indicates its use for upper respiratory catarrh, common colds, irritable coughs, hypertension and restlessness.

The German Standard Licence indicates lime flowers infusion for alleviation of cough irritation due to catarrh of the respiratory tract and for feverish colds for which a sweat treatment is desired.

Food Use

Lime flower is listed by the Council of Europe as a natural source of food flavouring (category N2). This category indicates that lime flower can be added to foodstuffs in small quantities, with a possible limitation of an active principle (as yet unspecified) in the final product. Previously, lime flower has been listed as GRAS (Generally Recognised As Safe) (Barnes *et al.* 2007).

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

According to the overview of the European market the following herbal preparations fulfil the criteria of the thirty years at least in Europe (a) while the herbal preparations (b) and (c) were found in literature references, and period of their use is longer than 30 years (BHP 1976).

Therefore for *Tilia* 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 evidence on traditional medicinal use is confirmed by a large number of publications providing consistent information.

a) Comminuted herbal substance for herbal tea preparation (In Germany since 1976, in Hungary since 1948, in Czechoslovakia since 1947)

From the literature (BHP 1976, Augustin *et al.* 1948, Bradley 1992; Blumenthal *et al.* 1998; Barnes *et al.* 2007, PDR 2007)

b) Liquid extract (DER 1 : 1) extraction solvent ethanol 25% (V/V)

c) Tincture (ratio of herbal substance to extraction solvent: 1 : 5), extraction solvent ethanol 45% V/V)

Herbal preparation in liquid dosage forms for oral use or Comminuted herbal substance as herbal tea for oral use.

Indications approved in the Member states:

Herbal medicinal product used as a diaphoretic in feverish colds (Germany, Poland).

Traditionally is used as an adjuvant for treatment of catarrhs of the upper respiratory tract and cold associated with dry, irritating cough (Czech Republic).

The traditional indication is: for the relief of early symptoms of common cold (Austria).

Traditionally used to promote sweating in the case of common cold, chronic coughing and catarrh and to relieve coughing (Hungary).

Traditional herbal medicinal product used as expectorant in dry cough associated with cold and to promote perspiration in case of fever (Lithuania).

The indication proposed by MLWP-HMPC:

1. Traditional herbal medicinal product used for the relief of symptoms of common cold.
2. Traditional herbal medicinal product used to relieve symptoms of nervous tension.

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

Posology

Adolescents, adults and elderly

a) Comminuted herbal substance

   Single dose 1.5 g, daily dose 3 - 6 g (Czech Pharmacopoeia 2009, supp. 2010)

   1.5g in 150 ml of boiled water 2 - 4 times daily (Blumenthal *et al.* 1998)

b) Liquid extract (1 : 1 in 25% alcohol) 2 - 4 ml daily (BHP 1976)

c) Tincture (1 : 5 in 45% alcohol) 1 - 2 ml daily (BHP 1976)

Based on literature data and information from member States the following posology and duration of use is proposed by HMPC/MLWP:

Posology

Adolescents, adults and elderly

a) Comminuted herbal substance

   Herbal tea: 1.5 g of the comminuted herbal substance in 150 ml of boiling water as a herbal infusion 2 – 4 times daily
   Daily dose 3 – 6 g

b) Liquid extract.

   2 ml 1 - 2 times per day
   Daily dose 2 - 4 ml

c) Tincture

Single dose 1 ml 1 - 2 times daily
Daily dose 1 - 2 ml

Duration of use

1. The therapy should start at first signs of common cold. 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.
2. If the symptoms persist during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

3. Non-Clinical Data

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

In vitro and animal studies

Differential cell growth effects on lymphocytes

Aqueous extracts or infusions obtained from the flowers of *Tilia* species are widely used for the treatment of anxiety in folk medicine. The antiproliferative action of aqueous, dichloromethane and ethanol extracts obtained from *Tilia cordata* Mill., flowers on tumoral (BW 5147 lymphoma) and normal lymphocytes was described. All extracts showed a selective action on tumoral cells, inducing apoptosis. In the case of normal T cells these extracts suppressed mitogen-induced proliferation without affecting viability, suggesting a suppressive but not cytotoxic effect. These effects were clearly concentration dependent. A coumarin (scopoletin), the main component in the dichloromethane extract presented antiproliferative action on BW 5147 cells, suggesting that it may be at least partly responsible for the activity displayed by this extract (Barreiro et al. 2006).

Sedative activity

The aqueous extract of *Tilia cordata* flowers showed *in vitro* stimulatory effects on lymphocyte proliferation (maximum stimulatory concentration: 20 µg/ml). This effect was mimicked by Ro 5-4864, an agonist of the peripheral benzodiazepine receptor, and by Pk 11195, an agonist/antagonist of the same receptor. These results suggest that *Tilia* extract could exert its stimulatory effect acting as an agonist of the peripheral benzodiazepine receptor (Anesini, et al. 1999).

Tilia cordata has been used in other sedative effects therapies include relief of sinus headache and migraines, insomnia, stress and panic disorders. It has been used also to treat nervous palpitations and has been also reported to lower high blood pressure caused by stress and nervous tension (Blumental et al. 1998). Lime flower's sedative effects have been reported as significant upon inhalation of *Tilia* sp. oil in mice (Blumental *et al.* 1998).

Antispasmodic – spasmogenic activity

In vitro, lime's seeds water extracts of *Tilia platyphylla* and *Tilia vulgaris* have been reported to exhibit antispasmodic activity followed by a spasmogenic effect on rat duodenum (Lanza and Steinmetz 1986).

The activity of selected constituents of lime flower (flavonoids and phenolic acids), were inhibited by atropine and papaverine, and reinforced by acetylcholine. The diaphoretic and antispasmodic properties claimed for lime flower have been attributed to p-coumaric acid and the flavonoids.

The effects of the ethanol extract of *Tilia cordata* Miller (*Tiliaceae*) were studied *in vitro* using intestinal smooth muscle cells of guinea pigs dispersed by collagenase. The extract induced a dose-dependent contraction of the dispersed smooth muscle cells. The obtained data indicated a direct effect of the *Tilia* cordata extract on the intestinal smooth muscle cells. In addition, the contractions induced by the extract were inhibited by atropine. These observations indicate involvement of some active constituents, with cholinergic properties, found in the alcohol extract to induce contraction of the intestinal smooth muscle cells *via* activation of the muscarinic receptors (Al-Essa *et al.* 2007).

Antimicrobial activity

Forty-one plant extracts, known in therapy for other properties, have been tested against nine fungal species Lime flower (*Tilia cordata)* has been documented to possess a range of antifungal activity (Guerin and Reveillere 1984).

Ethanolic bark extracts have shown interesting antimicrobial activity (MIC values against *Candida* 70mg/ml; against *Escherichia* and *Staphylococcus* 150 mg/ml) (Duke 1985).

Pancreatic lipase inhibition

Lipids are important components in human nutrition; however, their increased intake contributes to the development of obesity and can lead to multiple long-term complications. Pancreatic lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is a key enzyme for the absorption of dietary triglycerides. Interference with fat hydrolysis of ingested lipids, therefore inhibition of lipases decreases fat absorption. Extracts from 106 species of medicinal plants, vegetables and fruits were screened for potential lipase inhibitory activity. p-Nitrophenylpalmitate and 5-bromo-4-chloro-3-indoxylpalmitate were used as substrates in an *in vitro* test with crude porcine pancreatic lipase. Large-leaved lime (*Tilia platyphyllos*) extracts were the most active. Additionally, the activity of selected extracts with removed polyphenols was measured. The author concluded that extracts of large-leaved lime are a promising source for developing functional foods or isolating active compounds (Slanc *et al.* 2009).

Bioactivities of secondary metabolites from lime flower

Diaphoretic activity

The diaphoretic activity of the flowers of lime is suggested that is caused by quercetin, kaempherol and p-coumaric acid. Where "sweat cures" would be an advantage, lime tree has been used mainly for feverish colds and infections. It has been also used to reduce nasal congestion and relieve throat irritation and cough (Blumenthal *et al.* 1998, 2000).

Hepatoprotective activity

Only the methanolic extract from the flowers of *Tilia argentea* (lime tree) was found to show a hepatoprotective effect against D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced liver injury in mice. By bioassay-guided separation using *in vitro* D-GalN-induced damage to hepatocytes, five flavonol glycosides were isolated as the hepatoprotective constituents of the methanolic extract. Tiliroside, the principal flavonol glycoside which is also among the major constituents of *Tilia cordata*, strongly inhibited serum GPT and GOT elevations at doses of 25 - 100 mg/kg (p.o.) in D-GalN/LPS-treated mice. By comparing the inhibitory effects of tiliroside with those of its components alone, the

kaempferol 3-O-β-D-glucopyranoside moiety was found to be essential for the activity, and its effect was suggested to depend on the inhibition of tumour necrosis factor-α (TNF-α) production, decreased sensitivity of hepatocytes to TNF-α, and on the protection of hepatocytes against D-GalN (Matsuda *et al.* 2002).

Kaempferol-3-O-β-D-(6″-E-p-coumaroyl)-glucopyranoside (tiliroside) has been isolated from many natural plants sources. This compound showed very interesting pharmacological activities, e.g., cytotoxic for human leukaemic cell lines and anti-complement activity. However, there is a lack of data concerning the presence of this compound in medical plants. In this work, a reproducible and quick method was elaborated for the separation and determination of tiliroside in methanolic extracts of selected plants among which *Tilia* species. The content of tiliroside for various plant materials was compared (Nowak 2003).

Antidiabetic activity

A recent study on traditionally used medicinal plants, herbs and spices in Latin America included *Tilia platyphyllos* flowers. These were investigated to determine their phenolic profiles, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycaemia and hypertension. Lime flowers inhibited strongly both the α-glucosidase (exhibited the highest inhibition against α-glucosidase (≈100% at 2.5 mg of dried sample and its inhibitory activity was high even at lower dose 75%, at 0.5 mg of dried sample) and α-amylase enzymes (significant inhibition of porcine pancreatic α-amylase at the highest evaluated dose -71% at 25 mg of dried sample) and supposed to have potential for hyperglycemia and hypertension prevention associated with Type 2 diabetes (Ranilla *et al.* 2010).

Diuretic, sedative and antispasmodic effects

Tilia species are traditional medicinal plants widely used as sedatives and tranquilisers (Zhang, 2004). For this purpose, the infusion of their inflorescences is used to prepare a tea. In a study extracts of inflorescences from *Tilia tomentosa* Moench, one of the species found in the market, were purified using a benzodiazepine (BZD) binding assay to detect BZD receptor ligands in the different fractions. One of the ligands was identified as kaempferol, but it had low affinity (K(i) = 93 μM) for this receptor, and did not produce sedative or anxiolytic effects in mice. On the other hand, a complex fraction, containing as yet unidentified constituents, but probably of a flavonoid nature, when administered intraperitoneally in mice, had a clear anxiolytic effect in both the elevated plus-maze and holeboard tests, two well validated pharmacological tests to measure anxiolytic and sedative compounds. This active fraction had no effect on total and ambulatory locomotor activity. In conclusion, these results demonstrate the occurrence of active principle(s) like kaempferolin, at least in *Tilia tomentosa* while this can be found also in other *Tilia* species that may explain its ethnopharmacological use as an anxiolytic (Viola *et al.* 1994).

In addition, a number of actions have been associated with volatile oils including diuretic, sedative and antispasmodic effects in mice, which may also account for some of the reputed uses of lime flower (*Tilia cordata*) (Taddei *et al.* 1988, Barnes *et al.* 2007). Volatile oils are not thought to possess any true diuretic activity, but to act as a result of certain terpenoid components having an irritant action on the kidneys during renal excretion.

All these observations of such bioactivities help to account for some of the existing medical effects

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

No data on lime flower extracts have been found or reported.

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

Single-dose and repeated-dose toxicity studies

No data have been found.

Genotoxicity studies

Among six herbal infusions used worldwide (*Matricaria chamomilla*, ***Tilia cordata***, *Mentha piperita*, *Mentha pulegium*, *Uncaria tomentosa* and *Valeriana officinalis*) were assayed for anti-genotoxicity using the Somatic Mutation And Recombination Test (SMART) in *Drosophila melanogaster*. All these infusions are traditionally used for various medical purposes. Hydrogen peroxide was used as an oxidative genotoxicant to test the anti-genotoxic potency of the medicinal infusions. None of these infusions showed a significant genotoxicity, quite the reverse they were able to behave as desmutagens, detoxifying the mutagen hydrogen peroxide. The phenolic content of such herbal infusions is argued to be the possible scavenger of reactive oxygen radicals produced by the hydrogen peroxide (Romero-Jiménez *et al.* 2005).

No adequate genotoxicity studies carried out on lime flower in the scientific literature.

Carcinogenicity studies

No carcinogenicity studies carried out on lime flower in the scientific literature.

Reproduction and developmental toxicity studies

No reproductive and developmental toxicity studies carried out on lime flower in the scientific literature.

The safety of lime flower during pregnancy and lactation has not been established. In accordance with general medical practice, the herbal medicinal products (herbal teas or finished products) should not be used during pregnancy and lactation without medical advice.

3.4. Overall conclusions on non-clinical data

Lime flower has officially been recognised since 1947 in the first edition of the Czechoslovak Pharmacopoeia. In the current version of Czech Pharmacopoeia (2009, suppl. 2010). Also it was proposed since 1948 (Augustin *et al.* 1948) and then introduced in the VI Hungarian Pharmacopoeia in 1970 as an herbal remedy traditionally used in for the relief of symptoms in common cold (diaphoretic in feverish cold). Lime flower is also approved by the German Commission E Monograph, as well as in the British Herbal Pharmacopoeia (1976) I.e., it has been used as a traditional remedy for a long time without safety problems for much more than 30 years.

The published data referring to the indications and preparations is limited, but on the basis of existing data the pharmacological activities (BHP 1976; Madaus 1979; Brandley 1992) support the traditional use of *Tilia cordata*, *Tilia platyphyllos* and *Tilia x vulgaris* and preparations thereof in the proposed indication:

1. Traditional herbal medicinal product for the relief of symptoms of common cold
2. Traditional herbal medicinal product used to relieve symptoms of nervous tension

Since the efficacy of traditional herbal medicinal products is only plausible the safety must be guaranteed.

The lack of genotoxicity, carcinogenicity as well as reproductive and developmental toxicity studies does not allow the establishment of a Community List Entry.

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

No data available.

4.2. Clinical Efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

Antidiabetic activity

In this cross-sectional study was conducted by interviewing 310 diabetic patients visiting two medical centers in Jordan: Jordan University of Science & Technology Medical Center and Sarih Medical Center between December 2003 and August 2004. It is found that 31% of interviewed patients have used herbal products (96 patients). The results revealed that the most commonly used herbs by diabetic patients in Jordan were *Trigonella foenumgraecum* (22.9%), *Lupinus albus* (14.6%), *Allium sativum* (11.5%), *Allium cepa* (5.2%), *Nigella sativa* (7.3%), *Zea mays* L. (6.3%), *Urtica dioica* L. (8.3%), *Eucalyptus globules* LA (9.4%), *Olea europea* L. (3.1%), *Cumminum cyminum* (9.4%), *Coriandrum sativum* (10.4%), *Salvia officinalis* L. (3.1%), and ***Tilia cordata*** (1%). The side effects for all the assayed herbs, were reported by 36.5% of the patients and include headache, nausea, dizziness, itching, palpitation, and sweating. Among the patients, 72.9% used the herbs as adjunctive therapy along with their anti-diabetic drugs and 80.2% of the patients informed their physicians about their use. A 79.2% of the sample confirmed their intention to re-use these herbs as 86.5% of them were satisfied with their diabetes control. Therefore, it is essential to increase the level of awareness among diabetic patients and health care providers regarding the efficacy and toxicity of these medicinal herbs. (Otoom *et al.*2006)

There is a lack of clinical research, except the above referred reference by Otoom et al. 2006, assessing the effects of lime flower and rigorous randomised controlled clinical trials are required.

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

None reported.

4.3. Overall conclusions on clinical pharmacology and efficacy

Not specified by the Rapporteur.

5. Clinical Safety/Pharmacovigilance

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

There is a lack of clinical and preclinical safety and toxicity data for lime flower and further investigation of these aspects is required.

5.2. Patient exposure

No data available.

5.3. Adverse events and serious adverse events and deaths

The allergy to lime flower pollen (*Tilia cordata*) has been published several times and it is believed to be among the most common aeroallergens sensitisations (11.4%). The sensitisation to indoor aero-allergens, is similar in all age groups and it was less important than that of pollens (Loureiro et al.; Krakowiak *et al.* 2004; Mur *et al.* 2001).

It has been advised that lime flower should be avoided by individuals with an existing cardiac disorder (Duke 1985);as excessive use may result in cardiac toxicity, however, the scientific basis for this statement, if any, is not known (Barnes *et al.* 2007).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Special patient population

No data on use in children are available, therefore Tiliae flos can be intended only for adults.

Use in pregnancy and lactation

In the absence of data available and in accordance with general medical practice, it is recommended not to use the herbal medicinal products containing lime flower during pregnancy and lactation.

Overdose

No cases of overdose have been recovered in the scientific literature.

Drug abuse

No information in the literature search.

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

No data in the literature search.

5.6. Overall conclusions on clinical safety

In the absence of data available, Tiliae flos is intended only for adolescents, adults and elderly.

In the absence of data available and in accordance with general medical practice, it is recommended not to use the herbal medicinal products containing *Tilia* flos (lime flowers) during pregnancy and lactation.

Some cases of allergic reactions have been reported while the frequency is not known.

Moreover no adverse effects have been reported, showing enough safety data for the proposed traditional use of the herbal medicinal product.

Establishment of a Community List Entry it is not possible due to safety concerns, as there are no available data on genotoxicity, carcinogenicity and reproducibility on fumitory extracts.

6. Overall conclusions

The positive effects of lime flower on the relief of symptoms of common cold as well as of its activity to relive the symptoms of mild tension have been recognised empirically. The use is made plausible only by the existing *in vitro* and *in vivo* pharmacological data. There is a lack of controlled clinical studies with preparations containing lime flower.

In conclusion, Tiliae flos and its preparations can be regarded as traditional herbal medicinal products in the following indications:

- Traditional herbal medicinal product used for the relief of symptoms of common cold
- Traditional herbal medicinal product used to relieve symptoms of nervous tension

In the absence of available data, *Tilia* flos is intended only for adolescents and adults.

In the absence of data available and in accordance with general medical practice, it is recommended not to use the herbal medicinal products containing Lime flowers during pregnancy and lactation.

Some cases of allergic reactions have been reported while the frequency is not known.

Moreover no other adverse effects have been reported, showing enough safety data for the proposed traditional use of the herbal medicinal product.

As there no available data on genotoxicity, carcinogenicity and reproducibility on fumitory extracts the establishment of a Community List Entry, it is not possible due to safety concerns.

Annex

List of references