EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 March 2012
EMA/HMPC/239269/2011
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Fraxinus excelsior* L. or *Fraxinus angustifolia* Vahl, folium

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)	*Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl, folium
Herbal preparation(s)	Comminuted herbal substance
Pharmaceutical forms	Comminuted herbal substance as herbal tea (decoction or infusion) for oral use.
Rapporteur	Dezső Csupor
Assessor(s)	Noémi Tóth/Botond Lajos Borcsa

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.e ropa.eu **Website** www.ema.e ropa.eu

Table of contents

1. Introduction

Fraxini folium or ash leaf is by definition the dried leaf of *Fraxinus excelsior* L. or *F. angustifolia* Vahl (syn. *F. oxyphylla* M. Bieb). or of hybrids of these two species or of a mixture (Ph. Eur. 7.5, 2012).

Fraxinus is a genus of decidous trees of the olive family (Oleaceae) and consists of 43 accepted species, most of which are distributed in the northern hemisphere in two main distribution areas: North America (20 species) and Eastern Asia (20 species). In Europe and Western Asia, only three species occur. The genus is monophyletic and unique in the Oleaceae family by mostly having relatively large imparipinnate leaves and one-seeded samaras. The genus was first described by Linnaeus in 1753 and since then more than 450 species have been described, most of which are considered as synonyms today (Wallander, 2008). This high number is supported by the great variability in the leaf morphology (shape, texture, number of leaflets, leaflet margin, petiolule length, indumentum, epidermal papillae, rachis wings etc.), which can be regarded as a sign of individual adaptation towards environmental demands. Leaf morphology can also vary with the age of the individual specimens, thus this is a key factor for proper species and/or drug identification.

Fraxinus excelsior L. (synonym: *F. corariifolia*), known as bird's tongue, weeping ash, European ash, common ash or simply ash, is a tree with a maximal height up to 40 m. Its bark is grey, at first smooth but rough and finally fissured on old trunks. Its twigs and petioles are glabrous to densely pubescent, and its buds are black. 7-13 leaflets can be found in one leaf; these are oblong-ovate to oblong-lanceolate, long-acuminate, tapering to a rounded base, sessile, usually crenate-serrulate (the serrations more numerous than the lateral veins), villous on the midrib and towards the base beneath. Dimensions of leaflets are (30-)50-110 x 10-30(-40) mm. Rhachises vary from subglabrous to pubescent. The calyx and the corolla are absent and the anthers are dark purple. The wing of the fruits (samaras) is oblong-obcordance to lanceolate with the dimensions (20-)25-50 x (5-)7-10 mm (do Amaral Franco, 1972). This species is distributed from Northern and Central Europe to Western Russia (Wallander, 2008), and the borders of its distribution can be found in the Northern, Southern and Eastern continental margins (do Amaral Franco, 1972).

Fraxinus angustifolia Vahl (synonyms*: F. oxycarpa* Willd., *F. pallisiae* A.J. Willmott*, F. potamophila* Herder, *F. sodgiana* Bunge, *F. syriaca* Boiss.) or narrow-leafed ash is a tree with a maximal height of 25 m. Based on recent research results on the systematics of the genus *Fraxinus*, *F. oxyphylla* M. Bieb. is a non-preferred (illegitimate) synonym of *F. angustifolia* Vahl (Wallander, 2008). Its bark is grey and turns to finely and deeply reticulate-fissured. Its twigs and petioles are galbrous and its buds are dark brown. 5-13 leaflets can be found to form one leaf. These leaflets are oblong to linear-lanceolate, acuminate, cuneate and entire at the base, sessile or nearly so, the serrations usually as many as the lateral veins. The dimensions of the leaflets are 30-90(-100) x 8-25(-30) mm. The leaflets of juvenile trees are 7-15, obovate, obtuse to acute at the apex, and have the dimensions 8-30 x 5-17 mm. Rachis of this species are glabrous. The calyx and the corolla are absent. Samaras are oblong, glabrous, and the wing is oblong-obcordance to lanceolate. Samaras have the dimensions 20-45(-50) x 6-10 mm (do Amaral Franco, 1972). This species is distributed from Southern and Central Europe to Western Russia (Wallander, 2008), and it prefers river-banks, flood-plains and decidous woods as biotopes (do Amaral Franco, 1972).

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

- Herbal substance(s)

Dried leaf of *Fraxinus excelsior* L. or *F. angustifolia* Vahl or hybrids of these 2 species or of a mixture. It contains a minimum of 2.5 per cent of total hydroxycinnamic acid derivatives, expressed as chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (Ph. Eur. 7.5, 2012).

- Herbal preparation(s)

Herbal substance as herbal tea (as infusion)

Herbal substance as herbal tea (as decoction)

Powdered herbal substance in capsules

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

Not applicable.

- Constituents

Constituents isolated/detected/reported from *Fraxinus excelsior* L. leaf[1]

Simple coumarins

Considering the coumarin content of the dried leaves of this species, Carnat *et al.* (1990) found them present only in traces (0.01-0.05%). These compounds are represented in the herbal substance by: esculin, fraxin, (Paris & Stambouli, 1960) (Carnat *et al.*, 1990) (Iossifova *et al.* 1997); esculetin, fraxetin (Paris and Stambouli, 1960) (Iossifova *et al.* 1997); cichoriin (Murray *et al.*, 1982); scopoletin, isoscopoletin (Iossifova *et al.* 1997).

Iridoids

Very limited data are available on the occurrence of these compounds in the drug: deoxysyringoxide, hydroxynuezhenide, syringoxide (Gaedcke, 1993); deoxysyringoxidine (Gruenwald *et al.*, 2007) were reported to occur.

Secoiridoids

A larger variety of these compounds were isolated: 10-hydroxyligstroside, 7-β-D-glucopyranosyl-11-methyl-oleoside, oleoside-11-methyl-ester (Damtoft *et al.*, 1992); oleoside-7,11-dimethyl-ester (Damtoft *et al.*, 1992) (Iossifova *et al.*, 1997); excelsioside (Damtoft *et al.*, 1992) (Egan *et al.*, 2004); oleuropein, ligstroside (Damtoft *et al.*, 1992) (Iossifova *et al.*, 1997) (Egan *et al.*, 2004); Gl5 (Egan *et al.*, 2004).

Flavonoids

The qualitative quercetin and kaempferol glycoside composition of young and adult leaves was found to be similar (Tissut & Egger, 1972). From French samples, Carnat *et al.* (1990) measured the amount of flavonoids to be 1.4% in average, and including amongst these rutoside (= rutin) 0.5%, which was also identified in a very early study conducted by Paris and Stambouli (1960). According to Gaedcke (1993), the amount of these compounds can vary between 0.6-2.2%, from which 0.1-0.9% can be

[1] Due to the fact that with the herein extensively referenced review article of Kostova and Iossifova (2007) in certain cases it could not be clearly determined that given chemical components were isolated from the leaves or from other plant parts, only components with clearly identifiable origin are listed.

rutoside, but kaempferol-3-O-glucoside, quercetrin-3-O-glucoside, and their respective 3-O-rhamnoglucosides can also be found.

Triterpenes
Data on the occurrence of these compounds are also very limited: β-sitosterol, betuline, betulinic and ursolic acid (the latter in 0.7-2.5%) (Gaedcke, 1993).

Simple phenolic acids
The average amount of these compounds in the drug was found to be 3.2% (Carnat *et al.*, 1990), amongst which ferulic, caffeic, p-coumaric, p-hydroxybenzoic, protocatehuic, sinapic, syringic and vanillic acids can be found in the drug (Gaedcke, 1993).

Alkanes
Three of such compounds have been reported only by one source: hentriacontane, nonacosane, tetratriacontane (Gaedcke, 1993).

Other components
Mucilaginous compounds were found in amounts of 15.3% by Carnat *et al.* (1990), for which Gaedcke (1993) report a much broader interval from 9.5% to 22.2%. They also found 2.5% of tannins in their samples, for which Gaedcke (1993) give the range of 0.6-4%.

Carnat *et al.* (1990) also measured the content of mannitol as 21.0%, but for which Gaedcke (1993) give the range of 16.4-28.6%. The seasonal pattern of mannitol content was investigated by Oddo *et al.* (2002) in the leaves of both *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl growing in Northern Sicily. In the case of these species, it was found that mannitol content shows a gradual increase in spring, peaking in summer, which was followed by a gradual decrease. It was also concluded that rainfall was negatively correlated with the seasonal increase of mannitol content, which had its seasonal maximum value at the end of the dry season. This phenomenon can be regarded as the response of these species to drought conditions.

Minerals
The level of potassium was measured to be 1.7% by Carnat *et al.* (1990).

The aqueous dry extract contains approximately 5.7% phenolic components, while the aqueous-ethanolic (70% EtOH V/V) contains 8.8% (Gaedcke, 1993).

Constituents isolated/detected/reported from *Fraxinus angustifolia* Vahl leaf[2]

Coumarins

Limited data are available: esculin, esculetin, fraxin, fraxetin, scopoletin, isoscopoletin (Iossifova *et al.* 1997).

Secoiridoids
From the point of view of isolation, these are the most exhaustively targeted compounds reported from this species: ligstroside and oleuropein (Hosny *et al.*, 1991), (Hosny, 1998) (Çaliş *et al.*, 1993) (Çaliş *et al.*, 1996); fraxicarboside A [6''-*O*-*trans*-*p*-coumaroyl-10-hydroxyoleuropein], fraxicarboside B [6''-*O*-*trans*-caffeoyl-10-hydroxyoleuropein], fraxicarboside C [3"-*O*-cetyl-6''-*O*-*trans*-caffeoyl-10-hydroxyoleuropein], 10-hydroxyoleuropein, 10-hydroxyligstroside (Hosny, 1998); ligstral, angustifoliosides A [6''-*O*-(*β*-D-glucopyranosyl)-oleuropein] and B [6''-*O*-(*β*-D-glucopyranosyl)-ligstroside] (Hosny *et al.*, 1991) (Çaliş *et al.*, 1993) (Çaliş *et al.*, 1996); nuezhenide, angustifolioside C

[2] Due to the fact that with the herein extensively referenced review article of Kostova and Iossifova (2007) in certain cases it could not be clearly determined that given chemical components were isolated from the leaves or from other plant parts, only components with clearly identifiable origin are listed.

[6'-*O*-(*β*-D-glucopyranosyl)-neooleuropein] (Çaliş *et al.*, 1996); ligstrobutyl, oleobutyl, penta-*O*-methyl-ligstroside, tetra-*O*-methyl-oleoside-dimethylester (Çaliş *et al.*, 1996).

Lignans

Limited data available: (+)-pinoresinol-4'-*O*-*β*-D-glucopyranoside, (+)-fraxiresinol-1-*O*-*β*-D-glucopyranoside, (+)-1-hydroxypinoresinol-4'-*O*-*β*-D-glucopyranoside (Hosny, 1998)

Flavonoids

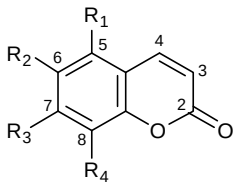
Limited data available: kaempferol-3-*O*-*β*-D-glucopyranoside, kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (Hosny, 1998)

Phenylpropanoids

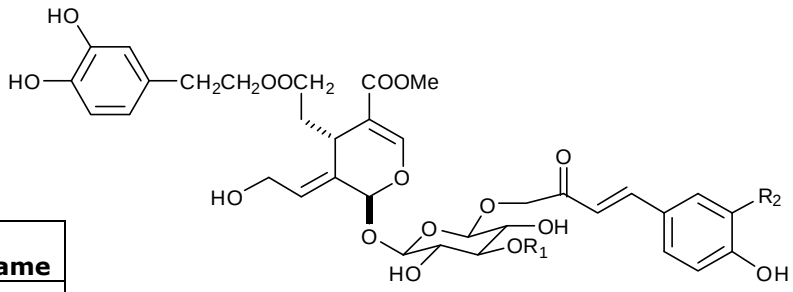
Verbascoside (= acteoside) (Hosny, 1998)

Simple phenolic compounds

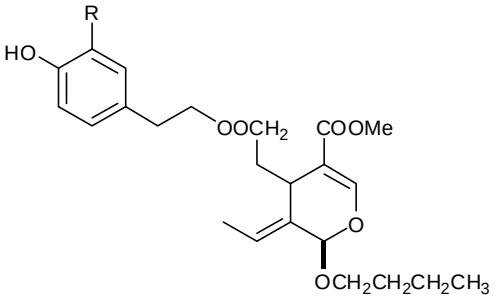
6-*O*-caffeoyl-*β*-D-glucopyranoside (Hosny, 1998)



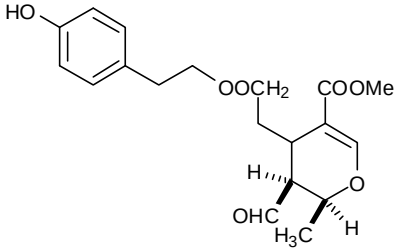
Coumarins				
R_1	**R_2**	**R_3**	**R_4**	**Trivial name**
H	OH	OH	H	esculetin
H	OGlu	OH	H	esculin
H	OMe	OH	OH	fraxetin
H	OMe	OH	OGlu	fraxin
H	OMe	OH	H	scopoletin
H	OH	OMe	H	isoscopoletin
H	OH	OGlu	H	cichorrin



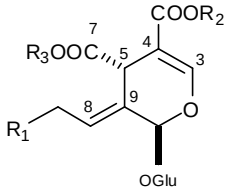
Secoiridoids		
R_1	**R_2**	**Trivial name**
H	H	fraxicarboside A
H	OH	fraxicarboside B
Ac	OH	fraxicarboside C



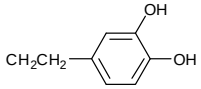
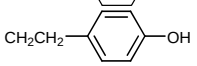
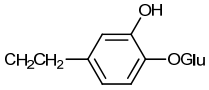
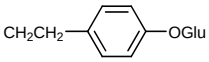
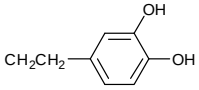
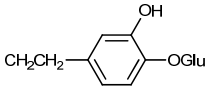
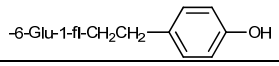
Secoiridoids	
R	**Trivial name**
OH	oleonutyl
H	ligstrobutyl

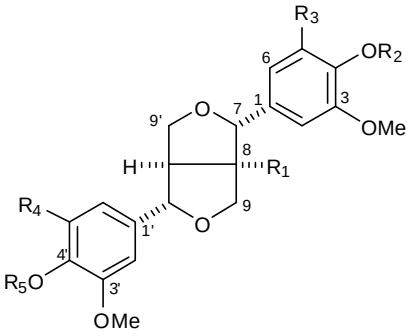


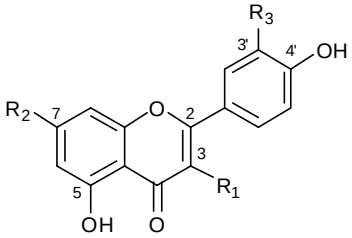
ligstral



Secoiridoids

R_1	R_2	R_3	Trivial name
H	Me		oleuropein
H	Me		ligstroside
H	Me		angustifolioside A
H	Me		angustifolioside B
H			angustifolioside C
H	Me		nuezhenide



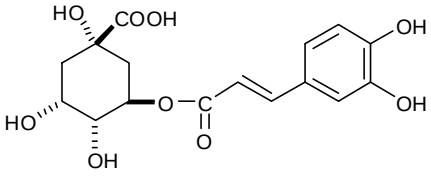


Flavonoids

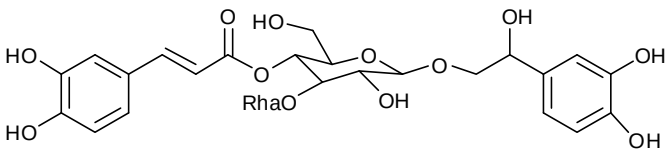
R_1	R_2	R_3	Trivial name
OH	H	H	quercetin
O-Glu^6-Rha	OH	OH	rutin
OH	OH	H	kaempferol

Lignans

R_1	R_2	R_3	R_4	R_5	Trivial name
H	Glu	H	H	H	(+)-pinoresinol-4′-*O*-*β*-D-glucopyranoside
OH	Glu	H	H	H	(+)-1-hydroxypinoresinol-4′-*O*-*β*-D-glucopyranoside



chlorogenic acid



Verbascoside

Figure 1. Main constituents of ash leaf

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

Spain

Both the powdered leaves and the herbal substance are on the market in Spain on the grounds of traditional use and in the form of four registered products since 1975 (for two herbal teas), 1987 and 1992. The first material (powdered leaves), is for oral capsules, the second (herbal substance) is for orally administered herbal teas for the preparation of either decoction or infusion.

Preparations

1) powdered leaves in capsules

since 1987

posology: 250-750 mg, 3 times daily

indication: arthritis and diuretic

2) powdered leaves in capsules

since 1992

posology: 350-700 mg, 2-3 times daily

indication: arthritis and diuretic

3) herbal substance as a tea (decoction)

since 1975

posology: 20 g/l divide in 2-3 cups a day

indication: arthritis and diuretic

4) herbal substance as a tea (infusion)

since 1975

posology: 10-30 g/l divide in 2-3 cups a day

indication: arthritis and diuretic

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Belgium	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Authorised products and as food available only in combinations, with an average number of combination substances of 2-3 (coated tablets) or >5 (multi ingredient herbal teas). Since 1963.

Member State	Regulatory Status				Comments
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	The herbal substance occurs on the national list (updated 01 April 2009) of ingredients may be used in food supplements, No registered or authorised products
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products

Member State	Regulatory Status				Comments
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Four preparation with traditional use, Herbal substance as tea (infusion or decoction) since 1975, also in combination products
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered or authorised products

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

Databases Science Direct, SciFinder, Pubmed and Web of Science were searched using the terms [*Fraxinus excelsior*], [*Fraxinus oxyphylla*], [*Fraxinus angustifolia*], [ash], [common ash] [narrow-leafed ash] and [leaf] and [extract]. Handbooks and textbooks on the topic were also used.

Data concerning Fraxini cortex, ash bark and other *Fraxinus* species (given either by scientific or by common names) were excluded.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Since ancient times, ash trees have been considered of a great value due to their rapid growth and hard timber. Their bark containing bitter components was used for tanning and as a colorant (Horánszky & Stohl, 1976). Due to some medicinal and healing thoughts and beliefs, it was also greatly respected in several European geographical regions and cultural circles. The ash sap was considered to be one of the most effective antidotes for snake bites (Horánszky & Stohl, 1976). Both the Norse and the Greek mythologies have their major chapters relating to ash trees (Jankovics, 1991), whereas these pieces of information are not linked to the medicinal use, therefore the detailed review of them is disregarded.

Concerning medicinal applications, the leaves and the bark of *Fraxinus excelsior* L. have been known as diuretic and rheumatic remedies since Hippocrates. From the beginning of the 20th century, the leaves of this species are mainly recommended against fever and rheumatism (Kostova and Iossifova, 2007).

In present times, Fraxini folium is an official drug of several pharmacopoeias including the Ph. Eur. (Ph. Eur. 6.0 2008; Ph. Eur. 7.5 2012).

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

Fraxinus species have been used in folk medicine in different parts of the world for their diuretic and mild purgative effects, as well as for treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp. The bark and the leaves of *F. excelsior* (and of *F. ornus*) are applied in the Bulgarian and Polish folk medicine against various diseases, including wound healing, diarrhoea and dysentery (Kostova & Iossifova, 2007).

Although the German Commission E listed this drug within the unapproved herbs (Blumenthal *et al.*, 1998), the drug is listed and described in different pharmacopoeias (Pharmacopée Francaise VIII, Pharmacopoea Helvetica V, Ergänzungsbuch zum Deutschen Arzneibuch 6. Ausgabe) (Gaedcke, 1993). The effectiveness of these claimed applications has not yet been documented (Blumenthal *et al.*, 1998). This statement is also in accordance with the categorisation of the PDR for Herbal Medicines (Gruenwald *et al.*, 2007), which also gives a list of the folk medicinal uses. Amongst these, ash leaf is used internally for fever, rheumatism, gout, oedema, stones, constipation, stomach symptoms and worm infestation; and externally for lower leg ulcers and wounds (Gruenwald *et al.*, 2007). Apart from these folk medicinal uses, the drug is also used to enhance urinary excretion in case of urinary stones (Gaedcke, 1993). According to Madaus (1938), *Fraxinus excelsior* was used in case of rheumatic disorders, gout, liver problems, jaundice, nephrolithiasis, kidney pain and oedema, cervical pain, conditions with fever, worm infestations, intoxications and as a laxative. Externally, the leaves are used for healing wounds and lower leg ulcers.

In traditional medicine, the herbal substance is still in use to enhance the urinary and digestive elimination functions, to facilitate the renal elimination of water, as an adjunct in weight loss programs, and for the treatment of minor pains in the joints (Bruneton, 1999). This high variety of folk and traditional medicinal applications is also supported by an overview prepared by Duke *et al.* (2002). All sources agree that the efficacy for the claimed applications has not been documented. Bruneton (1999) notes that since there are no known risks, the usage of this herbal substance should not be formally prohibited. Because the requirements for traditional use are fulfilled, according to the Directive 2001/83/EC as amended, the preparation of a traditional use monograph for ash leaf is justified. Posology for the herbal substance: 3-4 teaspoons (3.6-4.8 g) in water (Duke, 2002); 3 teaspoon leaf infused with 2 cups of water and to be consumed daily (Gaedcke, 1993).

Final indications for ash leaf proposed in the monograph for the traditional use

Indication 1)

Traditional herbal medicinal product used for relief of minor articular pain.

Indication 2)

Traditional herbal medicinal product used to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

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

The powdered herbal substance and the herbal substance for the preparation for herbal tea are marketed in Spain for the treatment of arthritis and as diuretic since 1975. Taken into consideration the evidence of the long-standing use (more than 30 years) of ash leaf and the authorisation dates of the below preparations, the inclusion of the following preparations and substances in a traditional use monograph is justified.

1) herbal substance as a tea (decoction)

since 1975

posology: 20 g/l divided in 2-3 cups a day

2) herbal substance as a tea (infusion)

since 1975

posology: 10-30 g/l divided in 2-3 cups a day

3. Non-Clinical Data

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

Pharmacodynamics

Antimicrobial activity

Extracts of the leaves of *Fraxinus excelsior* suppressed the growth of the fungi *Gloeosporum limetticola* and *Alternaria tennis* (Kostova and Iossifova, 2007). Aqueous extracts of the leaves of the same species showed strong inhibition on the growth of *Candida albicans* with an inhibition zone of 25 mm (Kostova and Iossifova, 2007).

Verbascoside was found to have antiviral properties (Kostova and Iossifova, 2007). This activity has also been described for other components, namely hydroxycoumarin derivatives esculetin and its diacetate against Newcastle disease virus replication in cell cultures (this testing was based on the results of an antiviral screen using picorna-, orthomyxo-, paramyxo-, and herpes viruses) (Galabov *et al.*, 1996). The cythopathic effect (CPE) inhibitory properties and the cytotoxicity activities were tested for esculetin and esculetin diacetate isolated from the stem bark of *Fraxinus excelsior*. In addition, purchased fraxetin and 7 other, semi synthetically substituted hydroxycoumarin derivatives were tested. The inhibitory concentrations were 36.0 and 62.0 µM, respectively, at 100 $CCID_{50}$/well (cell culture 50% infectious dose/well). These were inferior to the reference compound ribavirin, but while ribavirin at 2.0 mM/0.1 ml lacked the marked toxicity area, both esculetin and diacetate demonstrated it. The other components were inactive, therefore the authors concluded that methylation and glucosilation of esculetin lead to a loss of activity (Galabov *et al.*, 1996).

Complement inhibition and anti-inflammatory activity

The pure secoiridoid glucosides oleuropein and ligstroside were studied *in vitro* for their anticomplement action, as well as ability to prevent cobra venom-induced complement activation in normal human serum. Ligstroside has proven to be one of the most effective inhibitors of complement in guinea pig serum (IC_{50} 33 µg/ml), while the hydroxylated derivative oleuropein showed lower activity (IC_{50} 130 µg/ml). Therefore, based upon this observation (made with several further components, as well) the authors concluded that hydroxylation of secoiridoids at 10,2" or in the aromatic ring drastically decreases this activity (see also table on secoiridoids in Figure 1) (Ivanovska *et al.*, 1996) (Kostova and Iossifova, 2007).

Verbascoside (20 µM) was studied for its immunosuppressive action and multiplication inhibitory activity on human gastric adenocarcinoma cells (MGc80-3; in the latter study verbascoside was isolated from *Pedicularis striata* Pall (Jueyehesen)) (Kostova and Iossifova, 2007) (Sasaki *et al.*, 1989) (Ji *et al.*, 1997). In the experiment by Ji *et al.* (1997), the mitotic index declined from 48.5‰ to 29.5‰. The immunosuppressive action of verbascoside was studied by monitoring its hemolytic

plaque-forming cells (HPFC) inhibitory activity on mice. It was found to have a dose-dependent action in the range of 3-100 mg/kg body weight per os (Sasaki *et al.*, 1989).

cAMP-phosphodiesterase and lipoxygenase inhibitory activities

Scopoletin and fraxin (isolated from the bark of *Fraxinus japonica*) were found to have high inhibitory activity against cAMP-phosphodiesterase, while esculetin and fraxetin along with the lignan pinoresinol were also active (Kostova and Iossifova, 2007).

These previous coumarin compounds were also found to inhibit the formation of leucotrienes from arachidonic acid; therefore improve allergic conditions (Kostova and Iossifova, 2007).

Diuretic activity

Extracts from the leaves of *Fraxinus excelsior* are traditionally used to facilitate renal excretion. This diuretic activity is attributed to the presence of flavonoids. There are some data available on spray-dried powders prepared from the aqueous and ethanolic extracts made from the leaves of this species. These preparations caused a significant dose-dependent increase in the excretion of sodium and chloride ions, potassium and urea, and which were qualified as potentially useful medicinal products (Kostova and Iossifova, 2007).

This activity was the target of two animal studies performed on conscious rats. In the first study the acute effects of alcoholic and spray dried powers were studied by Casadebaig *et al.* (1989). It was found that aqueous extract showed no effect, but the alcoholic extract significantly increased the urinary sodium (UNa) concentration (Wright *et al.*, 2007).

Anti-hepatotoxic activity

Some coumarin and flavonoid components were found to possess liver protecting properties. These compounds: scopoletin, exerted pronounced choleretic activity; esculin, esculetin and isoscopoletin exhibited a cholagogue effect when administered to i.v. dogs; quercetin was found to increase bile secretion and the detoxifying function of the liver in experimental animals; and rutin showed low toxicity and marked choleretic effects when tested on rabbits, mice and rats (Kostova and Iossifova, 2007).

Cardiovascular effects

According to Çaliş *et al.* (1996), oleuropein and its derivatives have proven hypotensive and related cardiovascular activities, but since no exact data are available on the herbal substances (such as the secoiridoid content), the significance of these activities cannot be properly evaluated.

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

No data available.

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

Sensitising capacity of coumarins

Esculetin was proven to be of sensitising potency within the frame of studies performed on coumarins used in perfumery, cosmetics and therapeutic ointments (Kostova & Iossifova, 2007). Esculetin, esculin and isoscoloplamine showed a local photosensitising effect in an animal study (Masamoto, 2001). However, neither these coumarins nor any other similar structures were reported to be photosensitising after oral administration. Coumarin compounds detected so far in ash leaf do not possess the minimum structural requirements (a C-4 hydroxyl substituent and a C-3 non-polar carbon substituent) for anticoagulant activity (Barnes *et al.*, 2007). Taken into consideration that no report is

available on the sensitising potential of ash leaf, the relevance of the above described statement is not clear.

3.4. Overall conclusions on non-clinical data

Limited pharmacological data are available on ash leaf extracts and some constituents, mainly dealing with anti-inflammatory, diuretic and antimicrobial effects. Preclinical toxicological data are not available.

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 relevant data available.

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

No relevant data available.

4.2. Clinical Efficacy

No relevant data available.

4.2.1. Dose response studies

No relevant data available.

4.2.2. Clinical studies (case studies and clinical trials)

No relevant data available.

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

No relevant data available.

4.3. Overall conclusions on clinical pharmacology and efficacy

No relevant data are available on clinical research assessing the effects of ash leaf. The effectiveness of the traditional and claimed uses is not documented (Blumenthal *et al.*, 1998); therefore a monograph on a well-established use is not proposed. However, taken into consideration the general knowledge of its chemical components, some of its traditional applications seem to be plausible; e.g. anti-inflammatory and analgesic effects may be due to the complex action of flavonoids, coumarins, iridoids and secoiridoids, its laxative effect may be due to the action of mannitol; as this compound may also be responsible for its mild diuretic effects.

5. Clinical Safety/Pharmacovigilance

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

No relevant data available.

5.2. Patient exposure

No relevant data available.

5.3. Adverse events and serious adverse events and deaths

No relevant data available.

5.4. Laboratory findings

No relevant data available.

5.5. Safety in special populations and situations

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

5.6. Overall conclusions on clinical safety

No health hazards or side effects were recorded in conjunction with the proper administration of designated therapeutic dosages.

The presence of detected coumarin compounds is still controversial because one part of the literature reported these to be present only in traces, while the other – without proper quantitative information – reported these to be present in high variety and consequently assumed anti-inflammatory and analgesic properties (supposed inhibition of lipoxygenase and cyclooxygenase metabolism) (Bruneton, 1999).

6. Overall conclusions

The use of ash leaf has a long tradition in Europe. The provided clinical and non-clinical data do not fulfil the requirements of a well-established medicinal use with recognised efficacy and an acceptable level of safety.

Concerning medicinal applications, the leaves of *Fraxinus excelsior* L. have been known as diuretic and rheumatic remedies since Hippocrates. From the beginning of the 20th century, the leaves of this species are mainly recommended against fever and rheumatism (Kostova and Iossifova, 2007). Products containing Fraxini folium have been on the market for more than 30 years, thus they may be considered as traditional herbal medicinal products according to the criteria of 2004/24/EC Directive.

Taken into consideration the general knowledge of its chemical components, some of its traditional applications seem to be plausible; e.g. anti-inflammatory and analgesic effects may be due to the complex action of flavonoids, coumarins, iridoids and secoiridoids, its laxative effect may be due to the action of mannitol; as this compound may also be responsible for its mild diuretic effects.

Toxicological data on Fraxini folium is very limited. Due to the lack of data on acute and chronic toxicity, repeated dose toxicity, genotoxicity, mutagenicity, carcinogenicity, reproductive and

developmental toxicity, the safety of the therapeutic application of ash leaf cannot be substantiated. Nonetheless, neither the chemical composition nor the long-term widespread use in the European Union suggests that there is any risk associated with the use of ash leaf products, thus the traditional use can be suggested.

Annex

List of references