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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Citrus bergamia* Risso et Poiteau, aetheroleum

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)	*Citrus bergamia* Risso et Poiteau, fructus
Herbal preparation(s)	Essential oil
Pharmaceutical forms	
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Abbreviations

BEO	Bergamot Essential Oil
BEO-BF	Bergamot Essential Oil Bergapten-Free
BEO-HF/BF	Bergamot Essential Oil Hydrocarbon Fraction-Free and Bergapten-Free
BEO-MHF	BEO Monoterpene Hydrocarbons-Free
BEONVF	Bergamot Essential Oil Non-Volatile Fraction
BP	Bergamot Peel
CBSS	Children's Behavioural Style Scale
CH	Chitosan-based films
CH–BEO	Chitosan-based films containing BEO
CVD	Chronic Venous Disease
CVI	Chronic Venous Insufficiency
dl-TBOA	dl-threo-β-benzyloxyaspartic acid
DMSO	Dimethyl sulfoxide
EASI	Emotionality Activity Sociability and Impulsivity
ED50	Effective Dose 50% (dose effective in 50% of experimental animals)
EMA	European Medicines Agency
EPM	Elevated Plus-Maze
ESCOP	European Scientific Cooperative On Phytotherapy
HF	High Frequency
HMG	3-hydroxy-3-methylglutaryl
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HPLC	High Pressure Liquid Chromatography
HRV	Heart Rate Variability
HUVECs	Human Umbilical Vein Endothelial Cells
IFRA	International Fragrance Association
LC-MS	Liquid Chromathography coupled with Mass Spectroscopy
LD50	Lethal Dose 50%
LF	Low Frequency
LOX-1	Lectin-like oxyLDL receptor-1
MCAo	Middle Cerebral Artery occlusion
MIC	Minimal Inhibitory Concentration
MIC90	Minimal Inhibitory Concentration required to inhibit the growth of 90% of organisms

5-MOP	5-methoxypsoralen = bergapten
NF-κB	Nuclear Factor-κB
NMDA	N-methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
NVF	Non-Volatile Fraction
PSUR	Periodic Safety Update Report
RR	Relative Risk
SMC	Smooth Muscle Cell
SMD	Standardised Mean Differences
STAI	Spielberger State-Trait Anxiety Inventory
TLC	Thin Layer Chromatography
TNF-R	Tumour Necrosis Factor
VAS	Visual Analogue Scale

1. Introduction

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

- Herbal substance(s)

Citrus bergamia Risso et Poiteau belongs to the family Rutaceae, subfamily Esperidea. Trees grow to a height of 5 metres, with big dark green ovate leaves similar to those of lemon, fragrant star shaped white flowers and round yellow fruits.

The plant is native to tropical Asia but also found in Europe, for instance in Calabria, Italy. It grows also in Morocco, Iran and Ivory Coast (Di Donna *et al.* 2009). The trees are also cultivated on Kefalonia Island (Greece, Vlachata region) at the same latitude as in Reggio Calabria (Melliou *et al.* 2009) as well as in Sicily.

The plant appeared in Southern Italy before 1700 and it is believed that Christopher Columbus brought it from the Canary Islands to Italy.

C. bergamia is defined as a hybrid of bitter orange (*Citrus aurantium* L.) and lemon (*Citrus limon* L.) Burm. Fil., by some authors, or of *C. aurantium* L. and *Citrus aurantifolia* (Christm.) Swing. by others (Moufida *et al.* 2003). It is mainly cultivated for its essential oils that are obtained by rasping and cold pressing the fruit peel.

Bergamot is the common name of the fruit (*Citrus bergamia* Risso et Poiteau) and it has been believed that the name derives from the Italian city of Bergamo, placed in Lombardy, where the essential oil could have been first sold; however, this belief seems to be totally unfounded and due to the fruit and city names assonance.

The fruit peel is smooth and thin, whereas the pulp is slightly green–yellow, with an acidic and bitter taste. The peel of the pear-shaped fruit contains the essential oils and other bioactive constituents, whereas the juice is used for nutrition (Bergamot monograph Natural Standard).

- Herbal preparation(s)

Essential oil and juice are the preparations generally obtained from *Citrus bergamia*. Their chemical content has been extensively studied.

The juice obtained from the endocarp after essential oils extraction has been for a long time considered, also because of its bitter taste, just a secondary and discarded product of the essential oil production. For its organoleptic properties, bergamot juice has not reached the popularity of other citrus juices in the daily diet, but it is used to fortify fruit juice in place of synthetic additives (Di Donna *et al.* 2009).

Bergamot juice, as well as its peel, has attracted some attention because of its remarkable content of flavonoids. It contains different classes of flavonoids (e.g. flavanones and flavones) that can exert beneficial effects on human health (Gattuso *et al.* 2006).

Essential oil

Bergamot essential oil (BEO) is a greenish or brownish-yellow volatile oil with a bitter aromatic taste and a characteristic pleasant odour that made bergamot popular in cosmetics in the past and especially in aromatherapy in our days.

BEO is included in the official Pharmacopoeias of various countries. According to the Farmacopea Ufficiale Italiana (12th Ed.), BEO is obtained by cold pressing of the epicarp and, partly, of the mesocarp of the fresh fruit. Percentages of more characteristic components are reported in the Table 1.

Table 1. Percentage of single chemical components in Bergamot essential oil (Bergamotto essenza, Farmacopea Ufficiale Italiana 12th Ed.).

Chemical substance	**Interval ranges (%)**
α-pinene	0.2-0.7
sabinene	0.5-2.0
β-pinene	5.0-10.0
limonene	30.0-50.0
γ-terpinene	6.0-18.5
linalool	6.0-15.0
linalyle acetate	23.0-35.0
geranial	< 0.5
geranyle acetate	0.1-07
cariophyllene	0.2-05

BEO comprises a volatile (93–96% of total) and a variable percentage of a non-volatile (4–7% of total) fraction containing pigments, waxes, coumarins and psoralens (such as bergapten [5-methoxypsoralen (5-MOP)] and bergamottine [5-geranyloxypsoralen]) (Dugo *et al.* 2000). Volatile compounds, are monoterpenes, in particular 25-53% limonene, and high quantities of oxygenated compounds, such as linalool (2-20%) and linalyl acetate (15-40%).

The quality and quantity of the non-volatile fraction represent important parameters in terms of efficacy and safety for use of BEO and derived products for health purposes. While bergamot coumarins and psoralens show interesting bioactivities with therapeutic possibilities with, for example, anti-inflammatory, antianginal, and antiarrhytmic properties, the same chemicals have long been known to induce allergenic effects and severe skin diseases due to their photoreactivity (Guerrini *et al.* 2009).

The non-volatile residue contains about 0.2% bergapten (5-methoxypsoralen [5-MOP]) which is responsible for the photo-toxicity of BEO. Therefore, a bergapten-free essential oil (BEO-BF) together with an essential oil deprived of the hydrocarbon fraction and of bergapten (BEO-HF/BF) are prepared by extractive industries for perfumery and cosmetic uses (Bagetta *et al.* 2010).

The vacuum distillation of bergamot peels furnishes a high-quality essential oil that is totally bergapten-free. The oil obtained by vacuum distillation of the bergamot herbal matrix shows a composition quite similar to that of the cold-pressed oil (Belsito *et al.* 2007).

The most abundant compounds found in the volatile fraction are the monoterpene hydrocarbons limonene, γ-terpinene, and β-pinene, the monoterpene alcohol, linalool, and the monoterpene ester, linalyl acetate which, altogether, constitute more than 90% of the whole oil (Mondello *et al.* 1998).

The characteristic flavour and pharmacological properties of Citrus oils are mainly provided by the oxygenated compounds, which consist of alcohols, aldehydes, and esters, such as linalool, citral and linalool acetate, respectively. Linalyl acetate is considered the main constituent of the cold pressed BEO of the rind. Other important constituents are (–)-linalool, (+)-limonene and γ-terpinene. The linalool/linalyl acetate ratio can be approximately 0.38 %. The maximum sum of linalool + linalyl acetate has been found in the cold pressed BEO up to 55.8% (Melliou *et al.* 2009).

Oxygenated compounds, namely linalool and linalyl acetate, mark the flavour notes of BEO, whereas the hydrocarbon fraction does not have a fundamental role in determining the olfactory character of BEO. As compared with other citrus oils, bergamot oil is marked by a lower amount of limonene (25.6-53.0%) and higher amounts of linalool (1.7-20%) and linalool acetate (15.6-40.4%). Monoterpenes, such as limonene and pinene, do not contribute much to the flavour and are relatively unstable in heat and light, they are rapidly oxidized and can undergo hydration reactions and structural rearrangements; thus, it is necessary to remove them to increase the product's shelf life (Reverchon & Iacuzio 1997, Fang 2004).

Equal to other Citrus peels, Bermagot peel still contains other exploitable components in addition to BEO, such as pectins and flavonoids. The flavonoid profile of the peel consists of Citrus characteristic flavanone rutinosides and neohesperosides derived from naringenin, eriodictyol and hesperetin. In addition, a number of minor flavanone and flavone glycosides, not found in orange and lemon peels, have been identified (Mandalari *et al.* 2006).

The bio-variability of *Citrus bergamia* grown wild in Calabria (Italy) was investigated regarding the content of chemical markers (linalool, linalyl acetate and bergapten). The average marker content shows slight variations with the altitude and more evident changes with the latitude of the areas of plant collection (Statti *et al.* 2004). Mondello *et al.* studied extensively the coumarin and psoralen content of Calabrian bergamot oils using HPLC, finding interesting correlations between the content of the coumarins and the month of oil production and determining the variations of the enantiomeric distribution of some components during the whole production season (Mondello *et al.* 1998).

BEO is widely used by the cosmetic industries (e.g. in perfumes, soaps, body and sun tanning lotions) for its intense fragrance and freshness, being main ingredient of eau-de-cologne (Pernice *et al.* 2009). IFRA (International Fragrance Association) recommends a maximum of 0.4% bergamot oil in the final products for application to areas of skin exposed to sunshine. In order to obtain safer products, bergapten has to be removed or reduced to a level of 15 ppm in the final product (Costa *et al.* 2010).

BEO is not an alimentary product as such, but it is also widely used in food and confectionery industries as flavouring for liqueurs, teas, toffees, candies, ice creams, and soft drinks. It is also well-known for its use to flavour Earl Grey tea. In the pharmaceutical industries BEO is used as a flavouring for some medicinal products and as a cicatrizing agent, for its antiseptic and antibacterial proprieties while it is also used in aromatherapy (Costa *et al.* 2010, Martindale 2010).

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

Not applicable.

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

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	No product for internal use, but essential oil for aromatherapy
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products or not known
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Healing product for cutaneous use in fixed combination
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	No registered or authorised HMPs Essential oil used in aromatherapy Used as an excipient in MPs for cutaneous use
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:	
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:	

Member State	Regulatory Status				Comments
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

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

1.3. Search and assessment methodology

This assessment report reviews the scientific literature data available for *Citrus bergamia* from the Italian Pharmacopoeia monograph, from PubMed, internet, Medline as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration.

The keywords "*Citrus bergamia*", "bergamot", "bergapten" in all text fields were used and the electronic databases were searched till the end of July 2011.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

BEO has been used in Italian folk medicine since 1725, primarily for fever and worms. BEO has been used for mouth, skin, respiratory and urinary tract infections, gonococcal infections, leucorrhoea, vaginal pruritis. It has been also used for tonsillitis and sore throat. In 1804 Francesco Calabrò published a collection of folk remedies reporting for the first time that the topical use of BEO is considered to have wound healing effects (Pendino 1998).

In Hungary, a registered "healing product" in form of ointment containing a mixture of herbal preparations, including BEO, has been on the market since 2008 for mitigation of symptoms (erythema, infiltration, parakeratosis, urticaria) and for nursing of dry, pealing, squamosus skin in the mild or moderate psoriasis.

Promising research is continuing on the antibacterial, antifungal, and antioxidant properties of constituents in BEO.

Most recently, the interest in the ability of BEO to protect neurons from excitotoxicity has triggered an ongoing surge in neuroscience research.

BEO used for aromatherapy is known to be on the market in Bulgaria and in Italy.

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

For its antiseptic and antibacterial proprieties, BEO has been used in the folk medicine as antiseptic, to facilitate wound healing and as anthelminthic (www.bergamottoconsorzio.it). It has been included in some preparations for upper respiratory-tract disorders and hyperhidrosis (Costa *et al.* 2010, Martindale 2010).

BEO has been used in Italy since the beginning of 1900 for its disinfectant activity and as antiseptic for the skin. Bergamot essential oil terpenes-free has been used also for wounds healing. R.M. Gattefosse, in an Italian publication on “Therapeutic uses of bergamot essential oil” (Gattefosse 1932), describes the traditional uses for the following preparations containing BEO:

a) Liquid antiseptic for medical uses, local infections, personal hygiene

b) Solid disinfectant for daily hygiene

c) Ointment for disinfection of nasal cavities and mouth

d) Preparation intended to be converted into vapour for the treatment of respiratory diseases

Currently in preparations for cutaneous use the bergapten-free essential oil (BEO-BF) and the essential oil deprived of the hydrocarbon fraction and of bergapten (BEO-HF/BF) are used.

Likewise other essential oils, BEO is widely used in aromatherapy, and has recently received renewed popularity to improve mood and mild symptoms of stress-induced disorders such as anxiety, depression, behavioural disturbances in dementia and chronic pain (Halcon 2002, Costa *et al.* 2010, Martindale 2010, Bagetta *et al.* 2010) and to facilitate sleep induction (Wiebe 2000). Aromatherapy oils applied by inhalation do not appear to reduce anxiety (Graham *et al.* 2003), whereas aromatherapy massage has been shown to relieve symptoms of anxiety in patients with cancer (Bagetta *et al.* 2010).

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

R.M. Gattefosse (1932) reports the traditional use of BEO as follows:

a) Liquid antiseptic for medical uses, local infections, personal hygiene

   Add sodium sulforicinate (350-450 g) with 5 g of sodium borate and 445-545 g of distilled water.

   Twenty g of the preparation in one litre of water.

b) Solid disinfectant for daily hygiene

Bergamot essential oil	10 g
Boric acid	5 g
Sodium borate	85 g

   Reduce to powder and mix all ingredients. Dose for disinfection: 10 g to be dissolved in water before use.

c) Ointment for disinfection of nasal cavities and mouth

Frozen oil	85 g
Mineral or vegetable white wax	5 g
Bergamot essential oil	10 g

d) Preparation for vaporization and inhalation for the treatment of respiratory diseases

Bergamot essential oil terpenes-free 2.0 g

Crystallized menthol 0.2 g

Pour in a bowl of hot water and inhale the vapour generated.

No information on posology in the traditional use for inhalation is available.

The following posology was used in a clinical study in which BEO inhalation resulted as a method of relaxation: after dilution of the essential oil with distilled water 1:75, the mist is dispensed through a vaporizer placed approximately 60 cm away for 15 minutes for a single administration a day (Peng *et al.* 2009).

3. Non-Clinical Data

Antimicrobial activities

Antifungal effects

It has been reported that BEO exhibited antifungal activity against some dermatophytes and antibacterial activity against *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* (Karaca *et al.* 2007).

The *in vitro* activity of three BEO (essential oil, furocoumarin-free BEO and distilled BEO) against clinically relevant *Candida species* were investigated. In vitro susceptibility of 40 clinical isolates of *Candida spp.* (*Candida albicans*, n 5 20; *Candida glabrata*, n 5 13; *Candida krusei*, n 5 4; *Candida tropicalis*, n 5 2; *Candida parapsilosis*, n 5 1), associated with symptomatic and asymptomatic vulvovaginal candidiasis, was determined using a modification of the NCCLS M27-A2 broth microdilution method. MICs were evaluated for each of the oils alone and combined with sub-inhibitory concentrations of the well-known antiseptic, boric acid. To boric acid, all isolates had MIC values ranging from 0.094% to 0.187% (w/v). At 24 h readings, the MIC90s (for all isolates) were (v/v): 5% for essential oil of bergamot, 2.5% for the furocoumarin-free BEO, and 1.25% for the distilled BEO. At the 48 h reading, these values increased to >10%, 5% and 2.5%, respectively. At both readings, MIC90s for all oil-boric acid combinations were significantly lower than corresponding values for the oils alone ($P < 0.05$). It was concluded that the data indicate *in vitro* activity against *Candida spp*. of BEOs, suggesting their potential role for the topical treatment *of Candida infections* (Romano *et al.* 2005).

In another study, the activities of BEO, furocoumarin-free BEO and distilled BEO on dermatophytes such as *Trichophyton*, *Microsporum* and *Epidermophyton species* were investigated. *In vitro* susceptibility testing assays on 92 clinical isolates of dermatophytes (*Trichophyton mentagrophytes* n = 20, *Trichophyton rubrum* n = 18, *Trichophyton interdigitale* n = 15, *Trichophyton tonsurans* n = 2, *Microsporum canis* n = 24, *Microsporum gypseum* n = 1 and *Epidermophyton floccosum* n = 12) were performed using the CLSI M38-A broth microdilution method, except for employing an inoculum of 1–3 103 cfu/ml (colony forming units). MICs were determined at a visual endpoint reading of 80% inhibition compared with the growth control.
Results showed a MICs (v/v) of all fungi ranged from 0.156% to 2.5% for the essential oil, from 0.02% to 2.5% for the distilled BEO, and from 0.08% to 1.25% for the furocoumarin-free BEO. The three isolates of *T. tonsurans* and *M. gypseum* exhibited the highest MIC values.
It was concluded that data from this study indicate that BEO is active *in vitro* against several common species of dermatophytes, suggesting its potential use for topical treatment of dermatophytoses (Sanguinetti *et al.* 2007).

Chitosan-based (CH) films containing BEO at 0.5%, 1%, 2% and 3% w/w were prepared to evaluate their antifungal properties against *Penicillium italicum*. CH–BEO composite films showed a significant inhibitory effect on the growth of *P. italicum*, which depended on the BEO concentration. Chitosan films with the maximum BEO content (3:1 BEO–CH ratio) led to a total inhibition of the fungus growth during the first 5 days at 20°C. The antifungal effectiveness of the films decreased throughout the storage time (Sánchez-González *et al.* 2010).

Antibacterial effects

The effectiveness of oil and vapours of bergamot and its components against common foodborne pathogens was investigated. The disc diffusion method was used to screen oil and vapours against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157 and *Campylobacter jejuni*. The survival of each species, demonstrated to be susceptible in the *in vitro* studies, was tested on cabbage leaf for 60 s by direct contact and on chicken skin for 10 min by direct contact and 24 h by vapour. The results indicate that BEO was inhibitory and citral and linalool mimicked its effect ($P > 0.001$). Citral and linalool vapours produced 6 log reductions in *L. monocytogenes*, *S. aureus* and *B. cereus* populations on cabbage leaf after 8–10 h exposure but bergamot vapour exposure, while producing a similar reduction in *L. monocytogenes* and *B. cereus* populations, had no effect on *S. aureus*.

It was concluded that BEO was effective and linalool the most effective anti-bacterial component. Gram-positive bacteria were more susceptible than Gram-negative bacteria in vitro, although *Camp. jejuni* and *E. coli* O157 were inhibited by bergamot and linalool oils and by linalool vapour. All bacteria tested were less susceptible in food systems than *in vitro*. Of the Gram-positive bacteria tested *S. aureus* was the least susceptible to both the oils and the components tested. The results suggest the possibility that BEO, could be used as a way of combating the growth of common causes of food poisoning (Fisher & Phillips 2006).

Anti-inflammatory activity

Some of the components of BEO, limonene, linalool, linalyl acetate and alpha-pinene, have been shown to have anti-inflammatory effects. BEO was investigated for anti-inflammatory activity by using carrageenan-induced rat paw oedema test. For the anti-inflammatory activity measurement six different groups were established and BEO was administered in three different doses: 0.025, 0.05 and 0.10 ml/kg. Indomethacin was used as a reference agent. It was found that reduction in the inflammation was 95.70% with indomethacin, 27.56% with 0.025 ml/kg BO, 30.77% with 0.05 ml/kg BEO and 63.39% with 0.10 ml/kg BEO. Indomethacin showed the strongest anti-inflammatory activity among the drugs used. The strongest anti-inflammatory activity of BEO was seen with 0.10 ml/kg dosage. The median effective dose (ED50) value of BEO was found to be 0.079 ml/kg. The results indicate that BEO causes anti-inflammatory effects (Karaca *et al.* 2007).

Anxiolitic-like activity

A study was carried out to investigate the effect of BEO (1.0%, 2.5% and 5.0% w/w) administered to rats on both anxiety-related behaviours (the elevated plus-maze (EPM) and hole-board tests) and stress-induced levels of plasma corticosterone in comparison with the effects of diazepam. Inhalation of BEO (1% and 2.5%) and injection of diazepam (1 mg/kg, intraperitoneally) significantly increased the percentage of open arm entries on the EPM. The percentage time spent in the open arms was also significantly enhanced following administration of either BEO (2.5% and 5%) or diazepam. Total arm entries were significantly increased with the highest dose (5%), suggesting an increase in locomotor activity. In the hole-board test, 2.5% BEO and diazepam significantly increased the number of head dips. 2.5% BEO and diazepam attenuated the corticosterone response to acute stress caused by exposure to the EPM. In conclusion, both BEO and diazepam exhibited anxiolytic-like behaviours and

attenuated HPA axis activity by reducing the corticosterone response to stress (Saiyudthong & Marsden 2010).

Neuropsychopharmacological activity

The effects of BEO on the release of amino acid neurotransmitters in rat hippocampus have been studied by *in vivo* microdialysis and by *in vitro* superfusion of isolated nerve terminals. The BEO fractions employed are as follows: (1) BEO bergapten-free (BEO-BF) that corresponds to BEO deprived of bergapten and (2) BEO monoterpene hydrocarbons-free (BEO-MHF) that corresponds to BEO deprived of monoterpene hydrocarbons and bergapten. Intraperitoneal administration of BEO (100 µl/kg) significantly elevated the extracellular concentration of aspartate, glycine and taurine in a Ca^{2+}-dependent manner. A dose-relation study generated a bell-shaped curve. When perfused into the hippocampus via the dialysis probe (20 µl/20 min), BEO produced a significant increase of extracellular aspartate, glycine, taurine as well as of GABA and glutamate. The augmentation of all amino acids was Ca^{2+}-independent. Focally injected 1:1 diluted BEO preferentially caused an extracellular Ca^{2+}-dependent increase of glutamate. BEO concentration-dependently enhanced the release of [3H]d-aspartate from superfused hippocampal synaptosomes. Similar results were obtained by monitoring the BEO-evoked release of endogenous glutamate. At relatively high concentrations, the BEO-induced [3H]d-aspartate release was almost entirely prevented by the glutamate transporter blocker dl-threo-β-benzyloxyaspartic acid (dl-TBOA) and was Ca^{2+}-independent. At relatively low concentrations the release of [3H]d-aspartate was only in part (~50%) dl-TBOA-sensitive and Ca^{2+}-independent; the remaining portion of release was dependent on extracellular Ca^{2+}. Interestingly, the monoterpene hydrocarbon-free fraction of the essential oil appeared to be inactive while the bergapten-free fraction superimposed the releasing effect of BEO - supporting the deduction that psoralens may not be implicated. To conclude, BEO contains into its volatile fraction still unidentified monoterpene hydrocarbons able to stimulate glutamate release by transporter reversal and/or by exocytosis, depending on the dose administered (Morrone *et al.* 2007).

Neuroprotective activity

The effects of BEO and its fractions on excitotoxic neuronal damage have been investigated *in vitro*. The study was performed in human SH-SY5Y neuroblastoma cells line exposed to N-methyl-D-aspartate (NMDA). The fractions employed were bergapten-free extract of the essential oil (BEO-BF) and the essential oil deprived of hydrocarbon fraction and of bergapten (BEO-BF/HF). BEO and its fractions were diluted 1:10 in a 1:9 water/ethanol solution and then further diluted in culture medium to obtain final concentrations of 0.0005, 0.005 and 0.01%; identical volumes of ethanol were added to culture medium to investigate potential effects on NMDA-induced cell death. NMDA induced concentration-dependent, receptor-mediated, death of SH-SY5Y cells, ranging from 11 to 25% (0.25–5mM). Cell death induced by 1mM NMDA (21%) was preceded by a significant accumulation of intracellular reactive oxygen species (ROS) and by a rapid activation of the calcium-activated protease calpain I. In addition, NMDA caused a rapid deactivation of Akt kinase (a serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration) and this preceded the detrimental activation of the downstream kinase, glycogen synthase kinase 3 beta (GSK-3B). BEO (0.0005–0.01%) concentration dependently reduced death of SH-SY5Y cells caused by 1mM NMDA. In addition to preventing ROS accumulation and activation of calpain, BEO (0.01%) counteracted the deactivation of Akt and the consequent activation of GSK-3B, induced by NMDA. Results obtained by using specific fractions of BEO, suggested that monoterpene hydrocarbons were responsible for neuroprotection afforded by BEO against NMDA-induced cell death. Data demonstrated that BEO reduces neuronal damage caused *in vitro* by excitotoxic stimuli and neuroprotection was associated with prevention of injury-induced engagement of critical death pathways (Corasaniti *et al.* 2007).

In another study, performed by the same group of researchers, *in vitro* in human SH-SY5Y neuroblastoma cells exposed to NMDA, results obtained by using specific fractions of BEO, suggest that monoterpene hydrocarbons were responsible for neuroprotection afforded by BEO against excitotoxic NMDA-induced cell death associated with prevention of injury-induced engagement of critical death pathways (Corasaniti *et al.* 2007).

The effects of BEO on brain damage caused by permanent focal cerebral ischemia in rat have been investigated. BEO (0.1–0.5 ml/kg but not 1 ml/kg, given intraperitoneally 1 h before occlusion of the middle cerebral artery, MCAo) significantly reduced infarct size after 24 h permanent MCAo. The most effective dose (0.5 ml/kg) resulted in a significant reduction of infarct extension throughout the brain, especially in the medial striatum and the motor cortex as revealed by 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining of tissue slices.

Microdialysis showed that BEO (0.5 ml/kg) did not affect basal amino acid levels, whereas it significantly reduced excitatory amino acid, namely aspartate and glutamate, efflux in the frontoparietal cortex typically observed following MCAo. Western blotting experiments demonstrated that these early effects were associated, 24 h after permanent MCAo, to a significant increase in the phosphorylation and activity of the prosurvival kinase, Akt. Indeed, BEO significantly enhanced the phosphorylation of the deleterious downstream kinase, GSK-3β, whose activity is negatively regulated via phosphorylation by Akt. Conclusively, results showed that peripherally administered BEO could exert neuroprotection against ischemic injury (Amantea *et al.* 2009).

Hypolipidemic activity

Lectin-like oxyLDL receptor-1 (LOX-1) has been suggested to be involved in smooth muscle cell (SMC) proliferation and neo-intima formation in injured blood vessels. A study evaluated the effect of the non-volatile fraction (NVF), the antioxidant component of BEO, on LOX-1 expression and free radical generation in a model of rat angioplasty. Common carotid arteries injured by balloon angioplasty were removed after 14 days for histopathological, biochemical, and immunohistochemical studies. Balloon injury led to a significant restenosis with SMC proliferation and neointima formation, accompanied by increased expression of LOX-1 receptor, malondialdehyde and superoxide formation, and nitrotyrosine staining. Pretreatment of rats with BEONVF reduced the neointima proliferation together with free radical formation and LOX-1 expression in a dose-dependent manner. Results suggest that natural antioxidants contained in BEO may be relevant in the treatment of vascular disorders in which proliferation of SMCs and oxyLDL-related endothelial cell dysfunction are involved (Mollace *et al.* 2008).

Analgesic effects

The effects of BEO injected into the plantar surface of the hindpaw in the capsaicin test have been investigated *in vivo* in mice. The intraplantar injection of capsaicin produced an intense and short-lived licking/biting response toward the injected hindpaw. The capsaicin-induced nociceptive response was reduced significantly by intraplantar injection of BEO, while Sweet Orange (Citrus sinensis) essential oil was without effect. In contrast to a small number of pharmacological studies of BEO, there is ample evidence regarding isolated components of BEO which are also found in other essential oils. Among monoterpenes contained in BEO, the pharmacological activity of linalool has been examined. Following intraperitoneal administration in mice, linalool produces antinociceptive and antihyperalgesic effects in different animal models. The authors addressed the importance of linalool in BEO oil-induced antinociception (Sakurada *et al.* 2009).

In another study both linalool and linalyl acetate, injected into the hindpaw, showed a significant reduction of nociceptive response, which was much more potent than BEO. Intraperitoneal and intraplantar pretreatment with naloxone hydrochloride, an opioid receptor antagonist, significantly

reversed BEO- and linalool-induced antinociception. Pretreatment with naloxone methiodide, a peripherally acting μ-opioid receptor preferring antagonist, resulted in a significant antagonizing effect on antinociception induced by BEO and linalool. Antinociception induced by intraperitoneally or intrathecal morphine was enhanced by the combined injection of BEO or linalool. The enhanced effect of combination of BEO or linalool with morphine was antagonized by pretreatment with naloxone hydrochloride. Results provided evidence for the involvement of peripheral opioids, in the antinociception induced by BEO and linalool (Sakurada *et al.* 2011).

Cardiovascular properties

Coronaro-dilation and antiarhythmic activities of the furocoumarin bergamottine extracted from BEO have been investigated *in vivo* and *in vitro*. Bergamottine significantly decreased the typical electrocardiographic signs of coronary arterial spasm and the incidence of cardiac arhythmias induced by pitressin in anaesthetized guinea-pigs. Bergamottine also increased the dose of ouabain required to cause ventricular premature beats, ventricular tachyarhythmias and lethality. Bergamottine further reversed ouabain-induced persistent ventricular tachycardia and restored sinus rhythm in the guinea-pig. On isolated rat heart, bergamottine exerted a coronary dilator action and was able to reduce the hyperkinetic ventricular arhythmias caused by post-ischaemic reperfusion. Results indicate that bergamottine possesses antianginal and antiarhythmic properties and suggest that it is one of the active components responsible for the cardiovascular activity of non-volatile BEO (Occhiuto & Circosta 1996).

Hepatoprotective effect

BEO was investigated for its hepatoprotective effect on carbon tetrachloride-induced hepatotoxicity in rats. Six different groups were established. Silibinin was used as the reference agent. BEO significantly reduced the serum alanine transaminases (ALT) level when compared to CCl_4 group while it did not affect the serum aspartate transaminases (AST) level. The histopathological findings did not show any significant difference between the BEO and CCl_4 groups. The results suggest that BEO has a weak hepatoprotective effect in carbon tetrachloride induced acute liver toxicity (Karaca *et al.* 2005).

Haematologic effects

Chloroform bergamot fruit extracts and chemical standards corresponding to the main constituents detected were assayed for their capacity to increase erythroid differentiation of K562 cells and expression of γ-globin genes in human erythroid precursor cells. Three experimental cell systems were employed: (a) the human leukemic K562 cell line, (b) K562 cell clones stably transfected with a pCCL (a lentiviral vector) construct carrying green-enhanced green fluorescence protein under the γ-globin gene promoter, and (c) the two-phase liquid culture of human erythroid progenitors isolated from healthy donors. Results indicate that citropten and bergapten are powerful inducers of differentiation and γ-globin gene expression in human erythroid cells. On this basis, authors have suggested that data could have practical relevance, because pharmacologically mediated regulation of human γ-globin gene expression, with the consequent induction of foetal haemoglobin, is considered to be a potential therapeutic approach in haematological disorders, including β-thalassemia and sickle cell anemia (Guerrini *et al.* 2009).

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

Citrus bergamia essential oil has been investigated for its biological and pharmacological activities.

BEO possesses analgesic, anti-inflammatory, antibacterial and antimycotic activities. Linalool seems to be the most effective anti-bacterial component. Antibacterial activity was shown against *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus.* Gram-positive bacteria were more susceptible than Gram-negative bacteria in vitro. Antifungal activity has been shown against some dermatophytes.

BEO possesses anxiolitic and neuroprotective activity and attenuates HPA axis activity by reducing the corticosterone response to stress. Both essential oil and juice seem to have hypolipidemic effects.

The use of the essential oils in aromatherapy to improve mood, mild symptoms of disorders such as stress-induced anxiety, depression and chronic pain is thought to be therapeutically effective due to both the psychological effects of the odour and the physiological effects of the inhaled volatile components, where the latter are believed to act via the limbic system, i.e. the hippocampal formation, the hypothalamus and the pyriform cortex. Indeed, evidence exists indicating that aromatherapy can positively affect mood, alertness, and cognition and effects on alertness and relaxation have been noted in EEG patterns in studies of essential oils other than BEO (Bagetta *et al.* 2010).

Recently data have been gathered demonstrating that BEO is endowed with specific and reproducible effects on the CNS of rat. For systemic administration of increasing doses (100, 250 or 500 µl/kg, given intraperitoneally) BEO causes a dose-related sequence of sedative and stimulatory behavioural effects accompanied by increased energy in discrete frequency bands of the EEG spectrum (Bagetta *et al.* 2010).
These effects have been attributed to components of the volatile fraction of the BEO other than bergapten.

Pharmacological properties of bergapten (5-MOP) have been studied after UVA activation and in absence of UVA irradiation.
After UVA irradiation, 5-MOP can induce two types of photosensitisation: non oxygen dependent type III photosensitisation responsible for cycloaddition of 5-MOP to cellular DNA; oxygen dependent type II photosensitisation reaction.
In the absence of UVA irradiation, biological effects such as inhibition of melatonin catabolism and blocking of potassium channels have been shown (Forlot 2000).

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

In human volunteers the pharmacokinetics of single oral dose of 5-MOP (50 mg) have been studied. The absorption of 5-MOP is variable and it is positively influenced by the intake of food. Peak concentration of 5-MOP in plasma was about 235 ng and was reached within 2 hours, declining to 70% of this value by 12 hours and 60% by 24 hours. Means of 40-62 % and 38-48% were excreted with urine and faeces, respectively. 5-MOP is extensively metabolised and is subject to a first pass metabolism. The major components found in urine are glucuronic acid conjugate (Forlot 2000).

No pharmacokinetic data exist for the BEO.

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

No single/repeat dose toxicity, reproductive and developmental toxicity or local tolerance studies have been performed.

Phototoxicity

BEO causes phototoxic reactions and 5-MOP is considered the main phototoxic ingredient. The International Fragrance Association (IFRA, 1992) recommended a maximum of 0.4% of BEO in the final leave-on products for application to areas of skin exposed to sunshine to avoid phototoxic and photocarcinogenic hazard (Chouchi *et al.* 1996). In order to guarantee safety, it has been suggested to remove bergapten and other phototoxic components by distillation, the resulting essential oil being known as bergamot FCF (furocoumarin-free) or BEO-BF (bergapten free) and BEO-HF/BF (hydrocarbon fraction free; bergapten free) (Kejlovà *et al.* 2007; Corasaniti *et al.* 2007)

BEO is a widely used aromatic (fragrance) ingredient in cosmetics that may be applied on sun-exposed skin areas, although some components (bergapten, citropten, bergamotene and other furocoumarins) may cause phototoxic effects. BEO, when rubbed into the skin, sensitises it to Grenz rays, more to the harder than to the softer rays. A layer of oil on the skin has no sensitising effect, if not rubbed in. BEO alone does not irritate the skin. Residual hyperpigmentation from phototoxic reactions to BEO was described in 1916, and 5-MOP as the main phototoxic ingredient of BEO was isolated in 1938 (Oppenheim 1947).

To identify phototoxic effects, several fragrances (included BEO) were evaluated *in vitro* with a photohaemolysis test using suspensions of human erythrocytes exposed to radiation sources rich in ultraviolet (UV) A or B in the presence of the test compounds. Haemolysis was measured by reading the absorbance values, and photohaemolysis was calculated as a percentage of total haemolysis. Moderate phototoxic effects were induced by UVA in the presence of seven fragrances (benzyl alcohol, bergamot oil, costus root oil, alpha-amyl cinnamic aldehyde, laurel leaf oil, lime oil, orange oil) and by UVB due to incubation with five fragrances (alpha-amyl cinnamic aldehyde, hydroxy-citronellal, cinnamic alcohol, cinnamic aldehyde, laurel leaf oil). The authors concluded that BEO, as well as lime oil, orange oil and lemon oil caused moderate UVA-induced photohaemolysis (Placzek *et al.* 2007).

Genotoxicity

The genotoxic potential of bergapten (5-MOP) and BEO (containing equivalent amounts of 5-MOP) have been studied in haploid and diploid yeast (*S. cerevisiae*) by using solar simulated radiation (SSR). At equal doses of SSR, equal concentrations of 5-MOP alone or 5-MOP in BEO had a similar influence on survival and on induction of little cytoplasmic mutations, reverse and forward mutations, mitotic gene conversion, and genetically aberrant colonies including mitotic crossover. BEO contains psoralens which bind to DNA under ultraviolet exposure. A light exposure produces mono- and biadducts that are cytotoxic and highly mutagenic. However, in the dark, the oil seems not to be cytotoxic or mutagenic by itself (Averbeck *et al.*, 1990).

By using bergamot peel (BP) ethanolic extracts, an alcohol-insoluble residue was prepared as previously described and then used to obtain four liquid fractions. The genotoxic activity of the ethanolic bergamot extracts was studied in the SOS (group of cellular functions) chromotest (a bacterial test for detecting DNA-damaging agents), which employs the error-prone DNA repair pathway of *E. coli* PQ37, also known as the SOS response, a complex regulatory network that is induced by DNA-damaging substances. The test involves incubation of the bacteria with the sample under investigation and subsequent determination of β-galactosidase (β-gal) activity, because β-gal synthesis is used as a measure of SOS repair system induction. The activity of the constitutive enzyme alkaline phosphatase was used as a measure of protein synthesis and toxicity.

Each BP extract was dissolved in dimethyl sulfoxide (DMSO) and tested in triplicate. Their genotoxic activity was evaluated in comparison to that of the indirect-acting mutagen benzo[a]pyrene (B[a]P; 2.5 µg/assay) and the direct-acting mutagen 4-nitroquinoline-N-oxide (4-NQO; 0.02 µg/assay), used as positive internal controls.

The two BP extracts, used at doses up to 50 µg/assay, exhibit no genotoxic effect, also when undergoing enzymatic metabolisation. According to the authors this demonstrates that the BP extracts tested do not produce DNA lesions by blocking DNA synthesis and leading to SOS system induction. A lack of genotoxic effects due to bioactivation of the compounds in BP extracts used was confirmed by HPLC analyses, which showed identical chemical profiles for the extracts before and following exposure to the S9 mix (Trombetta *et al.* 2010).

Psoralen photoadducts have been measured in native DNA, bacterial cells, and in eukaryotic cells such as yeast, hamster cells, mouse cells, and human cells. In order to determine the genotoxic potential of bergapten, the genetic effects of 5-MOP and BEO (containing equivalent amounts of 5-MOP) were studied in haploid and diploid yeast (*Saccharomyces cerevisiae*) using solar simulated radiation. At equal doses of solar simulated radiation, equal concentrations of 5-MOP alone or 5-MOP in BEO have a similar influence on survival and on the induction of cytoplasmic “petite” mutations, reverse and forward mutations, mitotic gene conversion and genetically aberrant colonies including mitotic crossing over. No reciprocity was found between SSR dose and 5-MOP concentration for cytotoxic, mutagenic and recombinogenic effects. In the presence of chemical filters considerable protection was observed against the induction of genetic effects by 5-MOP and BEO containing 5-MOP in haploid and diploid cells. The protection was higher than expected from the light-absorbing properties, suggesting photochemical interaction. The protection is slightly higher for BEO than for 5-MOP. The induction of genetic effects by 5-MOP alone or BEO containing 5-MOP is independent of oxygen. Experiments on suction blister fluids taken from patients after topical treatment with BEO containing 5-MOP indicate that in comparison with water the bioavailability and thus the genotoxic effects of the compounds are decreased. Moreover, in addition to the filtering effect against the photoinduced genotoxic effects of BEO, the presence of chemical filters apparently reduces the penetration of BEO containing 5-MOP and provides a reduction in biological effectiveness (Averbeck *et al.* 1990).

Carcinogenicity

Most essential oils have been found to be cytotoxic without being mutagenic. However, some of their constituents may be considered as secondary carcinogens after metabolic activation. Psoralen, found in BEO, can induce skin cancer after formation of covalent DNA adducts under ultraviolet A or solar light (Bakkali *et al.* 2008).

3.4. Overall conclusions on non-clinical data

Citrus bergamia essential oil has been investigated for its biological and pharmacological activities. Bergamot essential oil possesses analgesic, anti-inflammatory, antibacterial and antimycotic activities.

BEO possesses also anxiolytic and neuroprotective activities. Both bergamot essential oil and juice seem to cause hypolipidemic effects.

Non-clinical studies indicate that the use of essential oil in aromatherapy can be effective to improve mood and symptoms of disorders such as stress-induced anxiety and chronic pain.

The toxicological properties of *Citrus bergamia* have not been adequately studied.

Mainly due to the presence of psoralens (e.g. 5-methoxypsoralen, 5-MOP), which are known to be phototoxic, genotoxic and possibly carcinogenic under UV and solar light, *Citrus bergamia* preparations may be suspected to pose similar risks. Linear furanocoumarins such as 5-MOP are phototoxic - their toxicity is enhanced in the presence of ultraviolet A radiation and they cause acute skin reactions generally manifested as itching, pigmentation and erythema. The cutaneous use of psoralen-free bergamot oil can be considered as safe having no risk of such skin reactions.

The genotoxic and mutagenic potential of BEO cannot be considered appropriately tested.

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 dose response studies available.

4.2.2. Clinical studies (case studies and clinical trials)

A study an open-label randomised controlled trial was carried out on 114 healthy undergraduate students at a university located in south Taiwan to investigate the effect of listening to soft music or inhaling *Citrus bergamia* aroma on the autonomic nervous system activity in young healthy individuals.

Participants were randomly allocated to one of four study groups including (1) a music group, (2) an aroma group, (3) a combined music and aroma group, and (4) a control group. Participants in the music group were asked to listen to preselected soft music for 15 minutes, and those in the aroma group were asked to inhale *Citrus bergamia* essential oil vapour generated from an ultrasonic atomizer for 15 minutes.

The essential oil was diluted 1:75 with distilled water, and the mist was dispensed through an ultrasonic atomizer placed approximately 60 cm away from the participant. BEO was an extract from the peel of bergamot orange (*Citrus aurantium* spp. *bergamia*). The combined music and aroma participants were given both music and essential oil for 15 minutes.

The outcome measure involved heart rate variability (HRV) indices measured before and after the intervention. The low frequency (LF) and high frequency (HF) components of the HRV were used to quantify modulation of the sympathetic and parasympathetic branches of the autonomic nervous system.

The percentage changes of normalised LF (p = 0.003), normalised HF (p = 0.001), and the ratio of LF to HF (p = 0.001) were significantly different among the four groups. Percentage change of normalised LF and HF were significantly different between the control group and the music group. For the percentage change of the ratio of LF to HF, the negative change in the music group, the aroma group, and the combined group was significantly different from that of the increase in the control group.

In addition, no significant differences were found in the percentage changes in systolic blood pressure, diastolic blood pressure, and mean heart rate in the four groups.

The Author concluded that listening to soft music and inhaling *Citrus bergamia* essential oil was found to be an effective method of relaxation, as indicated by a shift of the autonomic balance toward parasympathetic activity in young healthy individuals (Peng *et al.* 2009).

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

Though often lifesaving, stem cell transplantation (SCT) is a period of great distress for both child and parent. A double-blind, placebo-controlled randomised study evaluating the effect of the respiratory administration of BEO on the anxiety, nausea, and pain of 37 paediatric patients with malignant and non-malignant disorders undergoing stem cell infusion and their parents was carried out.

Patients were assessed at the time of recruitment, prior to infusion, upon infusion completion, and one hour post-infusion using the Spielberger State-Trait Anxiety Inventory (STAI) for parents and for children the STAIC, Children's Behavioural Style Scale (CBSS), visual analogue scale (VAS) for pain and nausea, and the Emotionality Activity Sociability and Impulsivity instrument (EASI) for children.

Children and adolescents in the treatment group, experienced greater anxiety ($p = 0.05$) and nausea ($p = 0.03$) one hour post-infusion. Reported pain in both groups was no longer significant one hour post-infusion. Parental anxiety declined in both groups but did not reach statistical significance. Child's monitoring coping style was significantly predictive of transitory anxiety post-infusion ($p = 0.01$).

It was concluded that the trial did not report a benefit of inhalation aromatherapy for reducing anxiety, nausea, or pain when added to standard supportive care, however, it provides the first experimental data on testing BEO among children and adolescents (undergoing stem cell infusion) (Ndao *et al.* 2010).

This study did not show any advantage using BEO in anxiety, nausea, and pain of 37 paediatric patients with malignant and non-malignant disorders undergoing stem cell infusion. However, because no toxic signals were detected, no particular contraindication in the paediatric population is indicated. BEO clinical use in children could be further investigated.

4.3. Overall conclusions on clinical pharmacology and efficacy

Inhaling *Citrus bergamia* essential oil together with listening soft music was shown to be an effective method of relaxation, in an open-label randomised controlled trial.

Another double-blind, placebo-controlled randomised study evaluating the effects of the respiratory administration of BEO on the anxiety, nausea, and pain of 37 paediatric patients (children and adolescents) with malignant and non-malignant disorders undergoing stem cell infusion did not reveal a benefit of inhalation aromatherapy for reducing anxiety, nausea, or pain when added to standard supportive care.

In conclusion, there are no sufficient data for the clinical use of bergamot essential oil for any indication.

5. Clinical Safety/Pharmacovigilance

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

5.2. Patient exposure

No data available.

5.3. Adverse events and serious adverse events and deaths

Freund, in 1916, was the first to describe a series of four cases of intense pigmentation in irregular areas after the use of Eau de Cologne followed by exposure to sunshine. He observed the same

phenomenon after experimental use of bergamot oil, one of the perfumes of Eau de Cologne. Rosenthal first used the term "Berlocque dermatitis" because of the form of the resulting pigmentation (Oppenheim 1947).

Hyperpigmentation of the neck, face, arms, or trunk in areas of light exposure have been attributed to psoralens in BEO derived from the peel of *Citrus bergamia* fruit. Cases have become much rarer since the introduction of artificial bergamot oil and the use of psoralen-free BEO.

BEO –rich in 5-MOP- has been used as a sun tanning agent for many years. In 1995, its use was banned from these products and limited to the treatment of certain skin disorders. Patients receiving PUVA therapy either ingest psoralens or apply them topically before exposure to UVA. Toxic reactions can occur due to overdosage with psoralen or UVA or accidental exposure to additional UVA, including natural sunlight exposure. The topical application of psoralens is more likely to induce phototoxic reactions. Second degree skin burns have been reported in two women who received PUVA (Herr *et al.* 2007).

Essential oil

BEO possesses photosensitive and melanogenic properties because of the presence of furocoumarins, primarily bergapten (5-MOP). Pure 5-MOP is also potentially phototoxic and photomutagenic. Despite its increasing application, there are only a few reports of phototoxic reactions to bergamot aromatherapy oil.

The cases of two patients have been described with localised and disseminated bullous phototoxic skin reactions developing within 48 to 72 hours after exposure to bergamot aromatherapy oil and subsequent ultraviolet exposure. The first case was of a woman that had used a bergamot aromatherapy oil preparation 3 days earlier and subsequently stayed outdoors for several hours on a sunny day. In the second case, a woman has visited a sauna 2 days previously where she was exposed to a bergamot aromatherapy oil preparation; after she was exposed to UVA radiation in an adjacent tanning cure. The skin lesions developed gradually within 48 to 72 hours. In both cases the patients have not used in parallel other creams and/or medications (Kaddu *et al.* 2001).

Essential oil in Earl Grey tea

A 44-year-old man had been drinking up to 4 l of black tea per day over the past 25 years. Since, his preferred brand had given him occasional gastric pain, he changed to Earl Grey. One week after the change, he noticed repeated muscle cramps. After 5 weeks of drinking tea, muscle cramps continued. Occasionally, he observed fasciculations, distal paraesthesias, and a feeling of pressure in his eyes, associated with blurred vision, particularly in darkness. Neurological examination confirmed reduced visual acuity and fasciculation. The patient assumed that there was a relation between his symptoms and his tea consumption and stopped drinking Earl Grey after 5 months, reverting to pure black tea again. Within 1 week, his symptoms had completely disappeared. He found that his symptoms did not recur as long as he consumed no more than 1 l of Earl Grey daily.

Earl Grey tea is composed of black tea and the BEO. Adverse effects of bergamot oil in this patient were explained by the potential effects of bergapten as a largely selective axolemmal potassium channel blocker, reducing potassium permeability at the nodes of Ranvier in a time-dependent manner. This may lead to hyperexcitability of the axonal membrane and phase alterations of potassium currents, causing fasciculation and muscle cramps. Impaired potassium channel function plays a pathogenic role in other disorders with fasciculation. Hyperexcitability may be enhanced by prolonged opening of voltage-gated sodium channels due to bergapten. BEO in Earl Grey tea, when consumed in excess, may induce muscle cramps, fasciculation, paraesthesia and blurred vision (Finsterer 2002).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No data available.

5.6. Overall conclusions on clinical safety

BEO possesses photosensitive and melanogenic properties because of the presence of furocoumarins, especially bergapten (5-MOP). 5-MOP is also potentially phototoxic and photomutagenic. Therefore in topical preparations, psoralen-free essential oil has been used in the last decades.

Even though the oil has been used extensively for many years, there are only a few reports of phototoxic reactions to bergamot aromatherapy oil, which are related to the contact with nebulized BEO only, because furocoumarins are not volatile. Localised and disseminated bullous phototoxic skin reactions developing within 48 to 72 hours after exposure to bergamot aromatherapy oil and subsequent ultraviolet exposure have been reported.

Since BEO could act as photosensitiser, it is generally suggested to avoid sun exposure in the hours following inhalation when psoralen-containing preparations are used and the method of inhalation does not exclude contact with the non-volatile fraction of BEO.

As a general precaution the use of psoralen-free BEO is recommended because of safety concerns related to furocoumarins (see 'Reflection paper on the risks associated with furocoumarins contained in preparations of *Angelica archangelica* L.' (EMEA/HMPC/317913/06)).

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

It has been reported that the presence of BEO in Earl Grey tea, when consumed in excess (more than 1 l per day) may induce reversible effects such as muscle cramps, fasciculation, paraesthesias and blurred vision.

6. Overall conclusions

Citrus bergamia essential oil, known also with the common name of bergamot oil (BEO), is traditionally used in folk medicine in particular in Italy. BEO in magisterial, handicraft and homemade preparations for cutaneous use, as an antiseptic for the disinfection of skin and as an aid in healing of minor wounds, has a long tradition of use in Italy that can be dated back since more than 30 years.

BEO is generally well tolerated, but it could act as photosensitiser, due to the presence of furocoumarins, mainly bergapten (5-metoxy-psoralen). Thus in the last decades a totally bergapten-free high-quality essential oil obtained through vacuum distillation of bergamot peels has been used.

However, adequate information on strength and posology used in these traditional preparations is not available and therefore a traditional use monograph cannot be established, due to the lack of sufficient data: the requirement laid down in Article 16a(1)(b) of Directive 2001/83/EC that the herbal substance or herbal preparation is "exclusively for administration in accordance with a specified strength and posology" is not fulfilled.

Recent literature data suggest that *Citrus bergamia* essential oil could be used by inhalation (aromatherapy) for the relief of mild symptoms of mental stress. However, this use is not substantiated by information on long-standing traditional medicinal use.

No single preparation medicinal product is authorised in the European Community and non-clinical and clinical studies are not sufficient to support any indication. Therefore a well established use monograph cannot be established because the requirement laid down in Article 10a of Directive 2001/83/EC that "the active substance has a recognised efficacy and an acceptable level of safety and that the period of well-established medicinal use has elapsed" is not fulfilled.

Annex

List of references