EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Viola tricolor* L. and/or subspecies *Viola arvensis* Murray (Gaud) and *Viola vulgaris* Koch (Oborny), herba cum flore

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)	*Viola tricolor* L. and/or subspecies *Viola arvensis* Murray (Gaud.) and *Viola vulgaris* Koch (Oborny), herba cum flore
Herbal preparation(s)	Comminuted herbal substance
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use or for infusion preparation for cutaneous use.
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1. Introduction

The herbal substance of wild pansy and/or European field pansy is mentioned in several well known handbooks such as Madaus (1938), Martindale (2007), Wichtl (1994; 2004), PDR for Herbal Medicines (2000; 2004), German Commission E Monograph, European Pharmacopoeia 6.0, Duke's Handbook of Medicinal Herbs (2002), Hänsel *et al*. (1994) and Chevallier Encyclopedia of Medicinal Plants (1996).

In the European Pharmacopoeia it is described as the dried flowering aerial parts of *Viola tricolor* L. (wild pansy) and/or spp. *Viola arvensis* Murray (Gaud.) and Koch (Oborny) (European field pansy). Wild pansy must contain at least 1.5 percent of flavonoids, calculated with reference to the dried substance, expressed as violanthin.

Wild pansy belongs to the family of *Violaceae*. The herb is distributed in Europe and Asia. Flowering aerial parts of wild pansy are harvested during summer.

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

- Herbal substance(s)

Not applicable.

- Herbal preparation(s)

Oral use:

3 g of the comminuted herbal substance/250 ml of water.

Dry extract: dry extract (6:1), extraction solvent: water.

Cutaneous use:

5 – 20 g of the comminuted herbal substance/litre of water

- 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

According to the information provided by the National Competent Authorities[1]

Czech Republic

Traditional Use

Is the Herbal Substance on the market? No

Combination products: Herbal teas (authorised since 1999)

Average number of combination substances: >5

What are the main combination substances? Flipendulae ulmariae herba, Equiseti herba, Harpagophyti radix, Salicis cortex, Solidaginis virgaureae herba, Callunae herba

Indications: as an adjuvant for inflammatory and degenerative diseases of locomotor apparatus (rheumatism, arthrosis, arthritis and gout), adjuvant therapy in flu like symptoms.

Denmark

Traditional use

Is the Herbal Substance on the market? No

Food supplements

Other information

Note that the *Viola tricolor* is accepted by the Food Agency in Denmark in food supplements (at least 100 mg).

Great Britain

Traditional use

Is the Herbal Substance on the market? Yes

Status: Registered products

Preparations: Dry aqueous extract (6:1), 62.5 mg per tablet (equivalent to 375 mg of herb)

Since when are the preparations on the market? > 40 years

Pharmaceutical form (Standard Terms): Dry aqueous extract (6:1), 62.5 mg per tablet (equivalent to 375 mg herb)

Posology: Tablets: 2 tablets/3 times daily

Combination products: The herbal substance is available in 2 combination products

Average number of combination substances: 2 - 3

What are the main combination substances? Burdock root

[1] The data are collected using the template entitled 'Document for information exchange for the preparation of the assessment report for the development of Community monographs and for inclusion of herbal substance(s), preparation(s) or combinations thereof in the list' (EMEA/HMPC/137093/2006)

Indications: Traditional remedy for spots, skin blemishes and dry eczema

Registration was granted in the old legislative frame, >40 years ago

Children: Not recommended

Contraindications: Known hypersensitivity to any of the ingredients

Poland

Traditional use

Is the Herbal Substance on the market? Yes

Status: Registered products

Pharmaceutical form (Standard Terms): Comminuted herbal substance for infusion

Since when are the preparations on the market? > 30 years

The herb is present in the market as single active ingredient only.

There are 10 registered herbal teas.

Posology

1) Oral use: 1 teaspoon or sachet (1.5g of herb) for tea preparation/
150 ml of hot water for 5 – 7 minutes
Adults and children over 12 years of age: 1 cup 3 times daily

2) Cutaneous use: 6 g (3 sachets) of herb for infusion in 250 ml of hot water for 5 – 7 minutes
Several times daily as a wet dressing

Indications: 1) Mild seborrhoeic skin condition, mild urinary tract complaints
2) Mild seborrhoeic skin condition

Additional comments: *Violae tricoloris herba* has been a subject of Polish Pharmacopoeia since 1937; recommended dosage in the last versions of the Polish Pharmacopoeia: single dose of herbal substance for oral use 3 g and for topical use 3 - 10%, daily dose for oral use 10 g. Posology: In mild seborrhoeic skin condition for cutaneous use. Oral use in mild seborrhoeic skin condition and as diuretic

Polish name of the herbal substance: ziele fiołka trójbarwnego.

Spain

Is the Herbal Substance on the market? Yes

Preparations: Comminuted herbal substance

Since when are the preparations on the market? 1991

Pharmaceutical form (Standard Terms): Comminuted herbal substance, capsules of 200 mg

Posology

Capsules: 1 or 2 capsules/3 times daily*

Indications: Dry cough or common cold

*Registration was granted in the old legislative frame, in 1991, not in the 2004/24/EC

Combination products: The herbal substance is available in 2 combination products

Average number of combination substances: 3 - 5

Posology for combination products: 1) dry cough or common cold
2) acne

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Only available in combination products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Food supplements
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:	Not present
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Not present
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
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:	Not present
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:	Not present
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	Only available in combination products
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

Databases assessed up to May 2010:	Science Direct, PubMed, Embase, Medline, Academic Search Complete, Toxnet
Search terms:	*Viola tricolor*, wild pansy, heartsease, and arvensis, vulgaris.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Traditional use

The traditional use of wild pansy goes back to ancient times. Wild pansy preparations were used during the Middle Ages mainly as a remedy for various skin ailments and were mentioned according to Madaus (1938) by Lonicerus 1564; Hieronimus Bock 1565, Matthiolus (1501-1577) and Andreas Caesalpinus (died 1602). Its therapeutic activity is presented in Madaus "Lehrbuch der Biologischen Heilmittel" (1938) and Jaretzky's "Pharmakognosie" (1937). The traditional use of wild pansy in different diseases has been thoroughly documented in several handbooks and in folk tradition (Allen and Hatfield 2004).

Skin disorders
Wild pansy was most often recommended for cutaneous and internal use in various skin disorders (Köhler 1883, Dragendorff 1898, Schimpfky 1900; Hoppe 1951, Hoppe 1958, Martindale 2009, PDR 2000; 2004; Chevallier 1996); especially those of a seborrhoeal nature (Martindale 2009), including weeping and dry eczema (Hänsel *et al*. 1994, Köhler 1883, Madaus 1938, Ożarowski 1976; Roeske 1955; Schöpke *et al*. 1993, British Herbal Pharmacopoeia 1996), impetigo, acne, and pruritus (Braun 1974, Wichtl 1994; 2004), also in irritation and vulvular itching, skin rashes and eruptions (PDR 2000; 2004, Hänsel *et al*. 1994)**,** and skin ulcers (Hoppe 1951, Hoppe 1958). Wild pansy was used in paediatric practice for treatment of various rashes and eruptions, eczema (Hoppe 1951, Hoppe 1958), and/or the treatment of seborrhea of the scalp of newborn infants (*crusta lactea*) (Rimkiene *et al*. 2003; Bundesanzeiger 1986). An ethnobotanical study indicates that the infusion of the whole plant (*Viola tricolor* L.) imbibed or applied externally is used in psoriasis (Amenta 2000).

Use for other purposes:

Respiratory tract disorders
In traditional medicine wild pansy was recommended internally, as expectorant (Hoppe 1951, Hoppe 1958; Kuźnicka and Dziak 1970; Muszyński 1954; Ożarowski 1976; Roeske 1955) for catarrhs of the respiratory tract (Hänsel *et al*. 1994, Rimkiene *et al*. 2003), coughs, whooping cough (Hänsel *et al*. 1994, Rimkiene *et al*. 2003) and in the treatment of acute bronchitis, inflammation of the throat and feverish colds (Hänsel *et al*. 1994; PDR 2000; 2004,Wichtl 1994; 2004).

Diuretic activity
Traditionally wild pansy is used as a diuretic (Hoppe 1951, Hoppe 1958, Bobowska *et al*. 1975; Kuźnicka and Dziak 1970; Muszyński 1954; Ożarowski *et al*. 1978; Wichtl 1994; 2004, Hänsel *et al*. 1994) in urinary tract disorders including cystitis and dysuria (Hoppe 1951, Hoppe 1958; Rimkiene *et al*. 2003).

Metabolic influence
In traditional medicine wild pansy is considered to be a "blood-purifying" remedy (Dragendorff 1898, Hoppe 1951, Hoppe 1958). It is recommended as diaphoretic (Bobowska *et al*. 1975; Wichtl 1994;

2004, PDR 2000; 2004) as well as for rheumatic complaints, gout, and arteriosclerosis (Hänsel *et al*. 1994; Hoppe 1951, Hoppe 1958; Kuźnicka and Dziak 1970; Muszyński 1954; Wichtl 1994; 2004).

Other indications
In traditional medicine wild pansy is also used in varicose ulcers, capillary fragility, haemorrhoids (Rimkiene *et al*. 2003; Hoppe 1951, Hoppe 1958) and gastrointestinal disorders (Hoppe 1951, Hoppe 1958). It was also proposed as a mild laxative (Dragendorff 1898, PDR 2000; 2002; Wichtl 1994; 2004).

Viola tricolor is contemporary used in folk medicine in Bulgaria as antitussive, diuretic, in skin disorders against dermatitis, against atherosclerosis and in Italy against psoriasis (Ivancheva and Stantcheva 2000; Leporatti and Ivancheva 2003).

Viola tricolor has been used for more than thirty years in Community, mainly as a remedy for various skin ailments, especially those of a seborrheal nature (Bradley 2006; Chevallier 1996; ESCOP 2009; Martindale 2009; PDR 2000; 2004).

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

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

Bundesanzeiger No 50: 13.03.1986, Violae tricoloris herba

External use: Comminuted herb: infusum 1.5g/cup of water, 3 times daily.

Hänsel *et al*. (1994)

Internal use: Comminuted herbal substance

- Tea: 1 g comminuted herbal substance in 1 cup (250 ml) of boiling water.
  Daily dose: 1 cup 3 times daily.
- Infusion for oral use: 5 - 10 g of comminuted herbal substance for 1 litre of boiling water.
  Use 1 dessert spoon 3 times daily.
- Tea: 1 g of comminuted herbal substance for 1 cup of boiling water.
  Drink 3 times daily after meal intake.
- Pulverised herbal substance: Half of teaspoon of pulverised herbal substance 3 times daily in hot sugar water.

Cutaneous use: For topical use: 4g of herb /150 ml of boiling water, also for bath additive.

Kraft and Hobbs (2004)

Wild pansy is used in skin inflammations (milk crust and mild seborrheal conditions). Wild pansy is also indicated in pruritus due to allergy, eczema and old age related itching, and itching associated with liver or kidney diseases.

Cutaneous use
One teaspoon comminuted herb in 1 cup of hot water for 5 minutes. Apply as a wet dressing to the affected areas of the skin 2 to 3 times daily.

For whole-body calming wash with wild pansy infusion: steep 3 tablespoons of herb in 1 litre of boiling water for 5 minutes and add 3 - 4 litres of cold water to maintain a water temperature 30 - 35 ^{0}C. Frequency: 3 times daily, depending on severity of the symptoms.

Martindale (2009)

Wild pansy flowering aerial parts (*Viola herba cum flore*) is used topically for minor skin disorders, particularly in seborrhoea. It is also used orally for gastrointestinal and respiratory tract disorders.

PDR for Herbal Medicines (2000, 2004)

Comminuted herbal substance for infusion, decoction. It is also available in ointments and shampoos for external use.

Tea for oral use: 1 dessertspoonful of comminuted herb for 1 cup (250 g) of boiling water (1 dessert spoon corresponds to 3 g of drug), or 1.5 g of herb for 150 g of boiling water.

An infusion for internal use is prepared using 5 - 10 g of herb/1 litre of water

This infusion is also used as a bath additive.

Daily dosage: Tea: 1 cup (250 ml) 3 times daily.

Pulverised herbal substance: Half of teaspoon of pulverised herbal substance 3 times daily in hot sugar water.

Cutaneous use: Rinses or wet dressings several times daily.

Polish Pharmacopoeia (1954, 1999)

Single oral dose: 3 g of comminuted herbal substance in decoction. Daily oral dose: 10 g.

Topical use: in infusions: 3 - 10%

2.4. Overall conclusion on the traditional medicinal use

Viola tricolor is a well known traditional herbal medicinal product used for centuries in Europe. Its medicinal use was described in many well known published manuscripts and textbooks.

3. Non-Clinical Data

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

Constituents of the aerial parts of *Viola tricolor* L. are received by Fraisse *et al*. 2001.

Flavonoids

The quantity of flavonoids in the herb *Viola tricolor* and *Viola arvensis* is 2.1% and 1.3% respectively (Manez and Villar 1989; Papp *et al*. 2004; Vincze-Vermes *et al*.1974). According to Ph. Eur. 2008 minimal amount of 1.5% flavonoids are expressed as violanthin (dried drug), principally rutin (quercetin 3-rutinoside), at one time called violaquercitrin (up to 0.8%) (Bandyukova and Sergeeva 1974; Carnat *et al*. 1998; Michaud *et al*. 1969; Kolos-Pethes 1965; Toker *et al*. 1998; Vincze-Vermes *et al*.1974), together with quercetin, luteolin and luteolin 7-glucoside (Boruch *et al*. 1985).

Other flavonoids: apigenin mono-*C*-glucosides: vitexin and isovitexin (saponaretin), luteolin mono-*C*-glucosides: orientin and isoorientin (Wagner *et al*. 1972), and scoparin (3'-*O*-methylluteolin 8-*C*-glucoside), saponarin (isovitexin 7-*O*-glucoside) (Hörhammer *et al*. 1965), apigenin di-*C*-glycosides including violanthin (apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-α-L-rhamnopyranoside), and four other - apigenin di-*C*-glycosides (one of them proved to be identical with vicenin-2) (Hörhammer *et al*. 1965, Wagner *et al*. 1972), violarvensin (apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-β-D-6-deoxygulopyranoside) (Carnat *et al*. 1998).

The highest concentration of rutin is usually found in the flowers (Hänsel *et al* 1994).

In a variety of *Viola tricolor* not used in therapy, flowers are reported to contain a remarkable amount 18 - 21 % of rutin. In other subspecies: *Viola tricolor* L. *var. maxima hort*. yellow flowers rutin concentration varies, in yellow flowers there is 15.96% of rutin and in white only 4.63% (Zielińska-Sowicka 1972). The other data (Gertig *et al*. 1966) present values of 4.5 - 10.5% in all flowers and in herb without flowers only 1.7% of rutin content.

Sixteen flavonoid glycosides have been separated from the methanol extract of wild pansy by micro-liquid chromatography: four flavonol *O*-glycosides - kaempferol-3-*O*-deoxyhexosyl (1→6) hexoside, quercetin-3-*O*-deoxyhexosyl (1→6) hexoside, isorhamnetin-3-*O*- deoxyhexosyl (1→6) hexoside and quercetin-3-*O*-deoxyhexosylhexoside-7-*O*-deoxyhexoside; nine flavone *C*-glycosides - luteolin-6-*C*-hexoside, chrysoeriol-6-*C*-hexoside, apigenin-6-*C*-pentoside-8-*C*-hexoside, apigenin-6-*C*-hexoside-8-*C*-pentoside, apigenin-6-*C*-deoxyhexoside-8-*C*-hexoside, apigenin-6-*C*-hexoside-8-*C*-deoxyhexoside, apigenin-6,8-di-*C*-hexoside, luteolin-6-*C*-deoxyhexoside-8-*C*-hexoside, luteolin-6-*C*-hexoside-8-*C*-deoxyhexoside; and three flavone *C*, *O*-glycosides - apigenin-X-*O*-hexoside-Y-*C*-deoxyhexoside-Z-*C*-hexoside, apigenin-6-C-deoxyhexoside-(6"-*O*-hexosyl-8-*C*-hexoside), apigenin-(6"-*O*-hexosyl-6-*C*-hexoside)-8-*C*-deoxyhexoside (Vukics *et al*. 2008).

Polysaccharides

The mucilage content in wild pansy herb is about 10%. Hydrolysis of polysaccharides results in glucose (35.1%), galactose (33.3%), arabinose (18.1%), rhamnose (8.4%), uronic acid (6.2%) and xylose (5.1%) residues (Franz 1969).

The water soluble fraction of polysaccharides is composed of glucose, galactose and arabinose residues (2:1.8:1.1) and galacturonic acid, rhamnose and xylose. The pectin fraction contains galacturonic acid, glucose, and galactose (Zabaznaya 1985, Hänsel *et al*. 1994). According to Deters, the polysaccharides of wild pansy are mainly composed of galactose, glucose, galacturonic acid (34:29:27), whereas arabinose, rhamnose and mannose are minor components (7:2:1) (Deters 2005).

Phenolic acids

The content is about 0.18%, including *trans*-caffeic, *p*-coumaric, gentisic, protocatechuic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, and vanillic acids (Komorowski *et al*. 1983, Boruch *et al*. 1985), and 0.06% to about 0.3 % salicylic acid and its derivatives, such as methyl salicylate and violutoside (violutin, glucosidoarabinoside of methyl salicylate) (Papay *et al*. 1987, Hänsel *et al*. 1994), and monotropitoside (primveroside of methyl salicylate) (Hänsel *et al*. 1994).

Volatile oil

The content is reported with 0.0086%, containing methyl salicylate as a principal constituent (Hänsel *et al*. 1994).

Carotenoids

In wild pansy flowers occurs *cis*-violaxanthin (Szabolcs and Toth 1970).
Yellow blossoms yield carotenoids (9.69 mg/g dry wt.), mainly 9-*cis*-violaxanthin (51.3%), all-*trans*-violaxanthin (29.6%), 13-*cis*- violaxanthin (1.7%), 15-*cis*- violaxanthin (0.6%), antherexanthin

(5.2%), 9,9'-antherexanthin (1.4%), lutein (0.39%), luteoxanthin (0.5%), *β*-carotene (1.4%), three di-*cis*-violaxanthins (Molnar and Szabolcs 1980), and four geometrical isomers of violaxanthins: 9,9'; 9,13'; 9,15- and 9,13-di-*cis*-violaxanthins (Molnar *et al*. 1986).

According to Hansmann and Kleinig (1982) the violaxanthin (about 75% of total carotenoids), occurs mainly as diesters, while monoesters and free violaxanthin are only present in trace amounts. The violaxanthin is esterified with the usual saturated acids (12:0, 14:0, 16:0, 18:0 acids) but also *β*-hydroxy polar 12:0, 14:0, 16:0 acids. In the latter three-esters-fraction, the two hydroxyl groups of violaxanthin are either both estrified with the usual acids, or one hydroxyl with a usual acid and one hydroxyl with *β*-hydroxy acid, or both hydroxyls with *β*-hydroxy acids, respectively. The minor xanthophylls: lutein, lutein epoxide and neoxanthin show corresponding ester patterns . Carotenoid esters are present in whole petals or lipid globules (plastoglobules) in which the esters are mainly localised.

Anthocyanins

Main pigment which is responsible for the violet colour of flowers of *Viola tricolor* is composed essentially of violanin (ca 33%), a derivative of delphinidin with D-glucose, L-rhamnose, *p*-coumaric acid, and 2.7 to 4% of potassium (Hayashi, and Takeda 1962, Takeda and Hayashi 1965, Hänsel *et al*. 1994).

Cyclotides (macrocyclic peptides)

Presence of violapeptide I, composed of 28 amino acids and showing hemolytic activity, in *Viola arvensis* Murray but not in *Viola tricolor*, was described in 1993 by Schöpke *et al*. Cyclotides (macrocyclic polypeptides), named vitri A and varv peptides A - H, have been first isolated from aerial parts of *Viola arvensis.* Each consists of 29 - 30 amino acids covalently cyclized via the amide backbone and by three disulphide bridges forming a cystine knotted arrangement (Claeson *et al*. 1998, Göransson *et al*. 1999; Trabi and Craik 2002; Xu *et al*. 2008).

According to Göransson *et al*. (2003; 2004), cyclotides expression profiles by LC-MS method, are almost identical in two closely related species *Viola arvensis* and *Viola tricolor*, and definitely different compared to *Viola cotyledon*, *Viola biflora*, *Viola riviniana* profiles.

The number of cyclotides in the *Violaceae* plant family is coming close in estimation to >9000 (Simonsen *et al*. 2005). They display a range of biological activities including uterotonic, haemolytic, cytotoxic, antimicrobial, anti-HIV, anti-fungal, anti-cancer, trypsin-inhibitory and insecticidal activities. They are probably involved in host defence function (Craik *et al*. 1999; Pelegrini *et al*. 2007). The structural features gives the cyclotides increased thermal, enzymatic, and chemical stability compared to linear peptides (Craik *et al*. 1999; Craik 2001; Hallock *et al*. 2000; Mulvenna *et al*. 2005; Svangård *et al*. 2004; 2007).

Other constituents

Tocopherols (30.2 mg% dry weight) in flowers (Baszyński 1961); coumarins with umbelliferone (Wichtl 1994; 2004) and xanthine derivatives (Schöpke 1993). Vitamin C content in fresh, flowering herb is 198 mg/100g, in dry herb 689.6 mg/100g (Hänsel *et al*. 1994), tannins (2.4 - 4.5 %), β-sitosterol and the triterpenes α-amyrin and β-amyrin acetate (Papay *et al*. 1987), nonsaturated fatty acids and calcium (Hänsel *et al*. 1994) and magnesium salts (Papay 1987).

Triacyl glycerols (57%) carotenoids and their esters (23%) are the main constituents and polar lipids, proteins, alkanes, phytol esters, plastid quinones and steryl esters have been detected in smaller amounts in plastoglobules isolated from petals of *Viola tricolor* L. (Hansmann and Sitte 1982).

Pharmacodynamics

***In vitro* experiments**

Spasmogenic activity

In a screening programme to evaluate several plants used in an Italian traditional medicine for gastrointestinal and respiratory tract complaints a hydromethanolic (methanol-water, 50:v/v) extract from *Viola tricolor* flowering tops was used. The extract at concentrations of 25 - 800 µg/ml exhibited spasmogenic activity on isolated guinea pig ileum muscles. The *Viola tricoloris* extract induced spasmogenic influence on circular muscle spontaneous contractions, and Ach-induced contractions and $BaCl_2$-induced contractions of longitudinal muscle (data not shown) (Izzo *et al*. 1996). Spontaneous contractions in the circular muscle were obtained with indomethacin.

Haemolytic activity

The reported presence of triterpene saponins in *Viola tricolor* herba from dry plant material (5.2% yield) with a haemolytic activity (*in vitro*; the haemolytic index IH=4000) (Tamas *et al*. 1981) was not confirmed in a later investigation (Schöpke *et al*. 1993, Hänsel *et al*. 1994). The haemolytic activity of *Viola arvensis* extracts is rather due to presence of the partly examined peptide violapeptide I (composed of 28 amino acids) isolated from *Viola arvensis* leaf (but not detected in *Viola tricolor*). Whether it is a cyclotide it is not completely clear (Schöpke *et al*. 1993, Wichtl 1994; 2004).

However, a case of haemolysis was presented in a 9 month-old child with a history of glucose-6-phosphate-dehydrogenase deficiency (G6PD) after intake of the half a cup of boiled extract of *Viola tricolor* (Behmanesh and Abdollahi 2002).

Cytotoxic activity

Three cyclotides, vitri A, varv A and varv E, isolated from aerial parts of *Viola tricolor* exhibited dose-dependent cytotoxic activity against two human cancer cell lines (U-937 GTB lymphoma and RPMI-8226/s myeloma) with IC_{50} values of 0.6 - 4 µM. The most potent activity was shown for vitri A. The three cyclotides tested had a narrow concentration range – less than 0.3 µM in 10 – 90% response interval in the log dose-response curves which points to a non-selective/non-specific effect (Svangård *et al*. 2004).

In other experiments Lindholm *et al*. (2002) described cytotoxic activity of cyclotides varv A and varv F obtained from *Viola arvensis* Murr. Their activity was tested in several human tumour cell lines and also in primary ovarian carcinoma cells obtained during surgical procedure and lymphocytic leukemia cells isolated from bone marrow or peripheral blood. Both varv A and varv F induced potent cytotoxic effect in tumour cell lines with IC_{50} values ranged from 2.73 – 7.49 µM. Cyclotide varv A presented potent cytotoxic activity in primary cultures of human tumour cells with selective toxicity to haematological chronic lymphocytic leukaemia cells (IC_{50} = 1.34 µM). The activity profiles of cyclotides were not correlated to the profiles of standard anticancer drugs: doxorubicin, vincristine, cytarabine, melphalan and topotecan. The authors suggest that the cytotoxic mechanism of action of cyclotides differs from that of the standard drugs used in cancer treatment.

Deters *et al*. (2005) showed significant reduction of proliferation but improvement of the cell viability of human keratinocytes *in vitro* with wild pansy oligo and polysaccharides. Tests were performed on primary human keratinocytes (NHK) cells and spontaneously immortalised cell line HaCaT. *Viola tricolor* pectin-like polysaccharides significantly inhibited DNA-synthesis of NHK and HaCaT cells at

concentration 10 μg/ml. However they induced mitochondrial dehydrogenase activity in viable NHK and HaCaT cells ($p<0.05$).

Antithrombin and anticancer activity

The methylene chloride and methanolic extracts of forty five plants of Russia, were used to determine antithrombin and anticancer activity, *Viola tricolor* L. var. *hortensis* included. Antithrombin activity of the methylene chloride extract of *Viola tricolor* was the most potent and displayed 93% activity, whereas the methanolic extract only exhibited 31% activity.

For cytotoxicity tests the mouse leukaemia cell line (L1210) was used. Again, the methylene chloride extract of *Viola tricolor* induced 79% inhibition of growth of leukaemia cells, whereas the methanolic extract inhibited the growth of 49% of cells (Goun *et al*. 2002).

Antioxidant activity

Mantle *et al*. (2000) compared relative antioxidant activities of British medicinal plants, *Viola tricolor* L. included. Antioxidative activity of the plants was tested through competitive scavenging of the $ABTS^{\bullet+}$ (2,2'-azino-bis-3-ethyl-benzthiazoine-6-sulphuric acid, presented in terms of mM Trolox equivalent – mMTE) or $O_2^{-\bullet}$ radicals (estimated as superoxide dismutase – SOD activity) *in vitro*. Fresh tissue was homogenised 1:10 (w/v) in 80% ethanol:20% water.

Antioxidant activity (mM TE/g dry weight) of fresh tissue *Viola tricolor* leaf was 1.46±0.32, whereas for flowers was 1.43±0.26. This activity was quite potent, as comparable extracts of *Ginkgo biloba* gave values of 0.62 and 0.61 mM TE/g dry weight, respectively.

Antibacterial activity

The infusion, decoction and ethanolic extract of *Viola tricolor* herb displayed significant inhibitory activity against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Candida albicans* and moderate activity against *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae.* The dichloromethane, ethyl acetate and methanolic fractions obtained by partitioning of Soxhlet of dried plant material, showed the lower activity.

The higher activity of the extracts containing complexes of components of the plant, relative to that of the fractions comprising compounds of different polarity, suggest a synergism in antibacterial action between compounds of wild pansy (Table 1, Witkowska-Banaszczak *et al*. 2005).

Table 1

Antimicrobial activity of infusion, decoction, ethanolic extract (EtOH) and dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), methanolic (MeOH) fractions of *Viola tricolor* herba (Witkowska-Banaszczak *et al*. 2005).

Microorganism	Infusion		Decoction		EtOH		CH_2Cl_2		EtOAc		MeOH	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram (+)												
Staphylococcus aureus	0.15	0.15	0.15	0.15	0.15	0.15	1.25	2.5	1.25	2.5	1.25	2.5
Staphylococcus epidermidis	0.15	0.31	1.25	1.25	1.25	1.25	1.25	2.5	2.5	5.0	1.25	1.25
Bacillus cereus	0.15	0.15	0.15	0.15	0.15	0.31	2.5	2.5	1.25	2.5	1.25	1.25
Enterococcus faecalis	5.0	5.0	5.0	5.0	2.5	5.0	2.5	2.5	1.25	2.5	1.25	1.25
Gram (-)												
Escherichia coli	2.5	5.0	2.5	5.0	2.5	5.0	2.5	5.0	2.5	5.0	2.5	5.0
Pseudomonas aeruginosa	1.25	5.0	1.25	1.25	1.25	2.5	2.5	2.5	2.5	5.0	1.25	2.5
Klebsiella pneumoniae	1.25	1.25	1.25	2.5	1.25	2.5	2.5	2.5	2.5	5.0	2.5	2.5
Fungi												
Candida albicans	0.15	0.31	0.31	0.31	0.15	0.31	1.25	2.5	2.5	2.5	1.25	1.25

[a]MIC (minimal inhibitory concentration, mg/ml), MBC (minimal bactericidal concentration, mg/ml).

MIC of standards - Gentamicin: Gram(-) and *Staphylococcus aureus* 2 μg/ml; *Staphylococcus epidermidis* 1μg/ml; *Bacillus cereus* 8μg/ml; *Enterococcus faecalis* 32 μg/ml. Nystatin: *Candida albicans* 16 μg/ml.

Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis and *Candida* albicans and moderate activity against *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae. The* dichloromethane, ethyl acetate and methanolic fractions obtained whilst the antibacterial activity is relatively weak this goes some way to supporting the traditional uses of wild pansy for skin conditions.

***In vivo* experiments:**

Influence on heart contractility.

Pápay *et al.* (1987) tested different fractions obtained from *Viola tricolor* in a model heart contractility in rats induced by intravenous vasopressin administration. Several extracts of wild pansy (ethyl alcohol, petrol-ether, ethyl acetate and n-butanol) significantly antagonised contractions of the heart and reduced ST segment elevation in electrocardiograms, which was comparable to activity of nitroglycerin and pindolol.

Skin condition

In an earlier experiment, eczema was induced in rats by prolonged feeding with rye (*Secale cereale*). After a diet augmented with fresh wild pansy given for 2 months (no information about the amount ingested), marked improvement of the skin condition was observed in comparison with control animals (Madaus 1938).

Diuretic activity

In another early experiment Vollmer and Weidlich (1937) showed no diuretic activity of Violae tricoloris herba. When administered orally to rabbits at 2.5 - 7.5 g/day or to mice at 50 - 200 mg/day wild pansy did not influence urine volume; in mice at the dose of 200 mg/day, however chloride excretion in urine increased by 108%.

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

Due to lack of data on pharmacokinetics no conclusions can be drawn.

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

There is no serious concern about toxicity of wild pansy herb.

Due to the lack of data on acute and chronic toxicity, repeated dose toxicity, mutagenicity, carcinogenicity, reproductive and developmental toxicity, a list entry for *Viola tricolor herba cum flore* cannot be recommended.

3.4. Overall conclusions on non-clinical data

Experimental preclinical data show antioxidant, antibacterial, diuretic, antithrombin and anti-inflammatory activity in skin conditions. Results of antimicrobial evaluation *in vitro* activity of *Viola tricolor* extracts support the traditional use of wild pansy even though the effects are relatively weak compared to standard antibiotics.

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

There are no data on human pharmacodynamics.

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

There are no data on human pharmacokinetics.

4.2. Clinical Efficacy

4.2.1. Dose response studies

There are no dose-response studies.

4.2.2. Clinical studies (case studies and clinical trials)

None were published on mono-preparations of wild pansy.

Combination products studies

Randomised, double-blind, vehicle controlled study of an ointment composed of *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica*.

The study was performed on 88 patients between 18 - 65 years of age with mild to moderate atopic dermatitis in dermatology practice. They were treated for 4 weeks with an ointment containing *Mahonia aquifolium, Viola tricolor* and *Centella asiatica* alcohol extracts (5g of each /100 g of ointment).

After 4 weeks of treatment the ointment reduced the primary (erythema, oedema/papulation, oozing/crust, excoriation and lichenification) and secondary endpoints (pruritus, global assessment of effectiveness and tolerability) slightly more than the base cream which was used as vehicle, but the difference was not statistically significant.

In this trial, an ointment could not be proven to be superior to a base cream with mild to moderate atopic dermatitis. However, a subanalysis indicated that the cream might be useful under conditions of cold and dry weather (Klövekorn *et al*. 2007).

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

No information available.

4.3. Overall conclusions on clinical pharmacology and efficacy

There are no clinical trials available on *Viola tricolor herba cum flore.*

5. Clinical Safety/Pharmacovigilance

There are no adverse effects reported from the Member States, however allergic reactions to *Violaceae* family should be considered.

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

There are no clinical trials available.

5.2. Patient exposure

None reported.

5.3. Adverse events and serious adverse events and deaths

Haemolysis was reported in a 9-month-old infant with G6PD deficiency given an extract of Wild pansy orally (Behmanesh and Abdollahi 2002).

Wild pansy belongs to herbs to be used with caution, due to salicylates content (Brinker 2001).

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

Intrinsic (including elderly and children) /extrinsic factors

The use of wild pansy is not recommended in children younger than 12 years of age due to lack of available data.

Patients with 6GTP deficiency can be at risk of haemolysis after *Viola tricolor* treatment.

Drug interactions

None reported.

Use in pregnancy and lactation

The wild pansy should not be used during pregnancy and lactation due to the lack of safety information.

Overdose

None reported.

Drug abuse, Withdrawal and rebound

None reported.

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

None reported.

5.6. Overall conclusions on clinical safety

There are no reports of adverse effects of wild pansy herb from Member States, however allergic reactions to *Violaceae* family should be considered.

Case of transient haemolysis in 9-months old infant confirms the approved use of *Viola tricolor* in adults and adolescents only.

6. Overall conclusions

The available data are sufficient to support the traditional uses of *Viola tricolor* herb in the European Community.

Viola tricolor herba cum flore fullfils the requirement of therapeutic use for at least 30 years (15 years within the community, Directive 2004/24/EC).

Indication:

Traditional herbal medicinal product for symptomatic treatment of mild seborrhoeic skin conditions.

Due to the lack of data on acute and chronic toxicity, repeated dose toxicity, mutagenicity, carcinogenicity, reproductive and developmental toxicity, a list entry for *Viola tricolor* can not be recommended.

Annex

List of references