



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

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

Rapporteur	W Dymowski
Peer-reviewer	A P Martins

HMPC decision on review of monograph on <i>Viola tricolor</i> L. and/or subspecies <i>Viola arvensis</i> Murray (Gaud) and <i>Viola vulgaris</i> Koch (Oborny), herba cum flore adopted on 25 November 2010	15 January 2020
Call for scientific data (start and end date)	From 1 April to 30 June 2020
Adoption by Committee on Herbal Medicinal Products (HMPC)	05 May 2021

Review of new data

Periodic review (from 2010 to 2020)

Scientific data (e.g. non-clinical and clinical safety data, clinical efficacy data)

- ☒ Pharmacovigilance data (e.g. data from EudraVigilance, VigiBase, national databases)
- ☒ Scientific/Medical/Toxicological databases
- ☐ Other

Regulatory practice

- ☒ Old market overview in AR (i.e. products fulfilling 30/15 years on the market)
- ☒ New market overview (including pharmacovigilance actions taken in member states)
- ☐ Referral
- ☒ Ph. Eur. Monograph

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☐ Other

Consistency (e.g. scientific decisions taken by HMPC)

☐ Public statements or other decisions taken by HMPC

☐ Consistency with other monographs within the therapeutic area

☐ Other

Availability of new information (i.e. likely to lead to a relevant change of the monograph)

<i>Scientific data</i>	Yes	No
New non-clinical safety data likely to lead to a relevant change of the monograph	☐	☒
New clinical safety data likely to lead to a relevant change of the monograph	☐	☒
New data introducing a possibility of a new list entry	☐	☒
New clinical data regarding the paediatric population or the use during pregnancy and lactation likely to lead to a relevant change of the monograph	☐	☒
New clinical studies introducing a possibility for new WEU indication/preparation	☐	☒
Other scientific data likely to lead to a relevant change of the monograph	☐	☒
<i>Regulatory practice</i>	Yes	No
New herbal substances/preparations with 30/15 years of TU	☐	☒
New herbal substances/preparations with 10 years of WEU	☐	☒
Other regulatory practices likely to lead to a relevant change of the monograph	☐	☒
Referrals likely to lead to a relevant change of the monograph	☐	☒
New / Updated Ph. Eur. monograph likely to lead to a relevant change of the monograph	☐	☒
<i>Consistency</i>	Yes	No
New or revised public statements or other HMPC decisions likely to lead to a relevant change of the monograph	☐	☒
Relevant inconsistencies with other monographs within the therapeutic area that require a change of the monograph	☐	☒
Other relevant inconsistencies that require a change of the monograph	☐	☒
<i>Other</i>	Yes	No
None identified	☐	☒

Summary and conclusions on the review

For the period 2010 to 2020, PubMed was searched on 25 August 2020, with the search terms »*Viola tricolor*«, »*Viola arvensis*« and »*Viola vulgaris*«. 46 publications were selected to be possibly relevant to the assessment giving priority to accuracy of search criteria. The search was verified by a screening with Google Scholar with a result of total 7170 publication hits for »*Viola tricolor*«. Searching »*Viola arvensis*« resulted in 1350 hits. In a second search, for the period 2010-2021, performed on 4 March 2021 (without language limits and priority to accuracy) »*Viola tricolor*« gave 7400 hits and »*Viola arvensis*« 15300 hits. »*Viola tricolor*« was used in a title of 189 of publications and »*Viola arvensis*« in the title of 62 publications. The term »*Viola vulgaris*« was found in 15 references, only referring to the HMPC monograph.

For the period 2010–2020 there were no published reports on adverse events caused by *Viola tricolor* or *Viola arvensis* herb or their preparations.

No references were provided by Interested Parties during the Call for data period.

During the review 46 new references not yet available during the first/previous assessment were identified as possibly connected with the area of the assessment and 22 references were considered to be relevant for the assessment.

None of the references justify a revision of the monograph.

The current EU herbal monograph mentions as possible source plants for *Violae tricoloris* herba cum flore *Viola tricolor* L. and/or subspecies *Viola arvensis* Murray and *Viola vulgaris* Koch (Oborny). Nowadays, only *Viola tricolor* L. and *Viola arvensis* Murray are taxonomically regarded as confirmed botanical species. *Viola vulgaris* (Oborny) Koch in contrast is simply considered as a synonym for *Viola tricolor* L. Accordingly, a monograph for Wild pansy, flowering aerial parts, (*Violae herba cum flore*) in The European Pharmacopoeia 10.0 (01/2008:1855, corrected 6.0) includes the two botanically confirmed species i.e. *Viola tricolor* L. and *Viola arvensis* Murray.

There are no new data/findings of relevance for the content of the monograph. Reference to the new pharmacopoeial monograph and modifications in the taxonomic status of source plants for *Violae herba cum flore* and subsequent naming should be adapted in the HMPC monograph and supporting documents when there is a need to revise the monograph.

No revision is considered required because there are no new data that will trigger a relevant change of the content of the monograph.

Scientific data

Clinical studies

For the review period, no new clinical studies relevant for *Violae tricoloris* herba cum flore were published. One report of Kirichenko *et al.* (2020) concerns clinical observations of a combination product (food supplement), containing *Violae tricoloris* herba 165 mg and equal parts of *Sambuci fructus* and *Calendulae flos* in comparison to placebo. It was tested on 60 male smokers with chronic obstructive pulmonary disease. Excluded were regular users of steroids or anti-inflammatory drugs. The endpoints were severity of symptoms, frequency of exacerbations, number of patients needing hospitalisation and spirometry tests. During the first 3 months of follow-up, there were no changes of clinical parameters. Over the next 6 months decreased insignificantly the symptoms in a BSS scale in the product group, as well as the number of exacerbations, the symptoms of cough, and the sputum production. The pulmonary functions (FEV1 and FEV1/FVC) were found increased. However, in the placebo group there was the same trend.

Assessor's comment:

The product contains 3 ingredients so that the possible effect cannot be attributed exclusively to Viola tricolor. Obstructive pulmonary disease needs regular supervision of doctor what doesn't meet the requirement for traditional herbal MPs to be used without medical supervision. The medicinal use of the combination in this indication is not well established within EU countries. In the report, neither randomisation nor blinding were described. The publication does not influence the reviewed monograph.

ESCP (2015) published a monograph on *Violae herba cum flore* for use in skin disorders such as eczema, seborrhoea, impetigo and acne, as well as cradlecap and nappy-rash of infants. Ershova and

Osipova (2016) reported a traditional use of the *Violae tricoloris* herba in acute respiratory viral infections in Ukraine. An infusion prepared of 2 tablespoons of the herbal substance in 0.5 l of boiling water, prepared in a thermos for the night is used for drinking during the next day. Goetz (2017) reported for an aqueous extracts of *Viola tricolor* herb in France the traditional use in phytotherapy, namely in furunculosis, as well as against Gram-positive and Gram-negative bacterias and *Candida albicans*. The herbal substance is used as an infusion of 1.5 g in a cup of boiling water, used 3 times a day. It is also used topically on a skin surface in a form of compresses using the infusion of 4 g herbal substance in a cup of boiling water.

Assessor's comment:

The publications on traditional use generally confirm the area of tradition in the EU »Traditional herbal medicinal product for symptomatic treatment of mild seborrheic skin conditions« as covered by the assessment report and does not influence the monograph content.

Non-clinical studies

Antibacterial, antiprotozoal potential

Khoshkam *et al.* (2016) tested a partially purified cyclotides fraction from *Viola tricolor* herb on an antibiotic activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The most sensitive strain for cyclotides was *E. coli*. *Viola* ethanol and butanol fractions were also more active against Gram-negative *E. coli* and *P. aeruginosa* while *S. aureus* was less susceptible. Gauttam *et al.* (2018) tested an 80% ethanol extract of *Viola tricolor* herb against *Propionibacterium acnes*. It had the lowest activity amongst other plant extracts, so it was excluded from further studies. Batiha *et al.* (2020) tested a methanolic extract of *Viola tricolor* (upper plant parts with flowers) and detected growth suppression of the protozoas *Babesia divergens*, *Babesia bovis*, *Babesia caballi* and *Theileria equi* *in vitro* and a weak inhibition of multiplication of *Babesia microti* in mice.

Assessor's comment:

Experiments on a concentrated cyclotide fraction can't be used directly and is, at the initial state of such knowledge, without influence on the monograph content. The ethanolic extract has a weak antibacterial activity against Propionibacterium acnes. The methanolic extract tested against Babesia strains exhibited a moderate activity in vitro, in vivo only at a high concentration. It does not affect the monograph.

Respiratory system

Harati *et al.* (2018) studied the effect of a dry extract of *Viola tricolor* flowers (100 g in 800 ml of 96% ethanol - water (1:1), on a mouse with asthma induced by OVA injection and inhalations. *Viola* extract (50 – 200 mg/kg) or dexamethasone 3 mg/kg influenced the cytokines IL-4 and IFN- γ excretion to the bronchoalveolar lavage fluid (BALF) and reduced the WBC count. The extract at higher doses decreased the IL-4 level versus asthmatic animals with increase of IFN- γ and reduced peribronchial inflammation at all doses; significantly only at 200 mg/kg.

Assessor's comment:

The area of the study is out of the scope of the monograph for traditional medicinal use in the European Union.

Pharmacological effects on CNS in mice

Ghorbani *et al.* (2012) observed that a dry extract from *Viola tricolor* herb (with 70% ethanol) prolonged pentobarbital-induced sleep in mice by 34% after administration of a dose of 300 mg/kg. Its ethyl acetate fraction increased the sleep duration by 51%. Mousavi *et al.* (2016) observed neuroprotective effects of the *Viola tricolor* extract on serum/glucose deprivation (SGD)-induced cell death test on PC12 cells *in vitro* at 25 µg/ml. The authors claim it could be at least in part attributed to antioxidant activity. Rahimi *et al.* (2019) found that pre-treatment with a *Viola tricolor* leaf extract (70% ethanol), its ethyl acetate and n-butanol fractions prolonged the latency to the first pentylenetetrazol (PTZ) induced generalized tonic-clonic seizures (GTCs) in mice. The hydroethanolic extract gave the effect only at 400 mg/kg; in contrast, the ethyl acetate fraction at all concentrations: 50, 100 and 200 mg/kg. The effect of comparator diazepam was markedly greater.

Assessor's comments:

The observed activities were weak and needed high dosages in animals. The area of the study exceeds the plausible use without medical supervision.

Sadeghnia *et al.* (2014) found that human breast cancer cells were susceptible for the ethyl acetate fraction of a *Viola tricolor* hydroethanolic extract. The fraction also significantly decreased the diameter of vessels on chicken chorioallantoic membrane CAM, with no influence on new formed vessels number.

Assessor's comment:

The observed effects are out of the scope of the monograph.

Saqib *et al.* (2020) observed a relaxant effect of a 70% ethanol extract from *Viola tricolor* herba on isolated rabbit aorta and paired atria preparations (*ex vivo*) and on a segment of the aorta, at concentrations of 5 mg/ml and 10 mg/ml. Its water fraction had a relaxant effect at 1 mg/ml and 0.1 mg/ml, the dichloromethane fraction at 10 mg/ml and 5 mg/mL (Verapamil, as comparator had relaxant effects at 0.1 µM and 0.03 µM).

In the experiment on anaesthetised rats *in vivo*, the ethanolic extract at doses of 1.0, 3.0, 10 and 30 mg/kg decreased the blood pressure by 18.7, 27.7, 42.3, 61.2 and 87.7 mmHg in a dose dependent manner. Likewise did carvedilol – a β -adrenolytic drug used as a comparator. In a further experiment *in vivo* on rats with left ventricular hypertrophy induced by isoproterenol, *Viola* hydroethanolic extract reduced the parameters 'weight of the heart to weight of the body' and 'weight of the heart to length of tibia'. It also reduced significantly the plasma renin concentration. NO and CGMP were markedly increased. In the experiment with acute myocardial infarction induced by isoproterenol, *Viola* extract, partially lowered cardiac enzyme markers compared to the intoxicated group. Carvedilol substantially reduced enhanced levels of cardiac enzyme markers. The histopathological review of the group treated with *Viola* extract revealed less necrosis, edemas and inflammatory cells than in the isoproterenol treated group. The effect was estimated comparable to the carvedilol group.

Assessor's comment:

The use of the preparation in cardio-vascular indications exceeds the medical application plausible without medical supervision as included in the monograph.

Pharmacovigilance data

The WHO VigiBase, reported for the years 2010-2020 two cases on *Viola tricoloris* herba in France and Great Britain. First case (2016): a 46 years old man treated for abscess, took products with powdered *Bardanae radix* and *Viola tricoloris* herba for 3 days and experienced pruritus and acne

(pimples). In this non-serious case, the *Viola tricolor* ingredient has a status of 'suspected'. The second case was serious: a man (31 years old) treated for psoriasis with a high dosage multicomination of 37 herbs for 121 days. As a result he was admitted to the hospital in Britain (2010) with symptoms of nausea, jaundice and epigastric pain, with a suspicion of drug-induced hepatitis or heavy metal poisoning. The patient used daily 20-40 dosage forms of every product a day.

Assessor's comment:

In the first case, Bardanae radix is known to be used for promotion of secretory activity. In the second case, the patient used 37 herbal preparations of unknown quality in high doses, thus, no conclusion on a single preparation can be drawn. The cases does not influence the content of the monograph for Viola tricoloris herba cum flore.

Quality

During the period 2010-2021 works on wild pansy flavonoids were continued by Gonçalves *et al.* (2012), and Wianowska *et al.* (2017). Schumacher *et al.* (2011) and Piana *et al.* (2013a, 2013b) elaborated a flavonoid-rich extract preparation and a method of rutin quantification in the extract. Furthermore data on a content of cyclotides in wild pansy herb was published by Tang *et al.* (2010). In 2015, a book chapter published by Göransson *et al.* (2015) summarises the knowledge on *Viola* cyclotides. Hellinger *et al.* (2015) followed peptide analysis by mass spectrometry and found 164 cyclotides in *Viola tricolor* plant. Jafari *et al* (2017) further worked on optimisation of *Viola tricolor* cyclotides extraction.

Assessor's comment:

New quality data are not relevant for the revision of the content of the EU herbal monograph.

Regulatory practice

New market overview

From a new market overview, up to August 2020, there were no new products identified on the market that could trigger a revision of the monograph.

References

a) References relevant for the assessment:

Exclusion criteria used:

- Reviews on cyclotides and cyclic peptides and their potential applications
- Other species or varieties taxons not covered by the current European Pharmacopoeia monograph
- Botanical, plant physiology, ecological, publications,
- Publications on edible plants and functional food

Batiha GES, Beshbishy AM, Alkazmi L, Adeyemi OS, Nadwa E, Rashwan E, *et al.* Gas chromatography-mass spectrometry analysis, phytochemical screening and antiprotozoal effects of the methanolic *Viola tricolor* and acetonc Laurus nobilis extracts. *BMC Complementary Medicine and Therapies* 2020, 20: 87

Ershova IB, Osipova TF. Fitoterapija ostrich respiratornykh virusnykh zaboilewaniy. Fitoterapija Vid A do Ja [Herbal Medicine from A to Z]. *Aktual'naja infektologija* [Actual Infectology] 2016, 4 (13): 73-81 [Russian]

ESCOP Monograph *Violae herba cum flore*. Wild Pansy (Flowering Aerial Parts). Online series 2015. Available at: www.escop.com

Gauttam VK, Munjal K, Negi N. Bioactivity guided fractionation of potent antiacne plant extract against *Propionibacterium acnes*. *African Journal of Biotechnology* 2018, 17 (13): 458-465

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Goetz P. Phytoaromatherapie de la furunculose. *Phytotherapie* 2017, 15: 297-299

Gonçalves AFK, Friedrich RB, Boligon AA, Piana M, Beck RCR, Athayde ML. Anti-oxidant capacity, total phenolic contents and HPLC determination of rutin in *Viola tricolor* (L.) flowers. *Free Rad Antiox* 2012, 2 (4): 32-37

Göransson U, Malik S, Slazak B. Cyclotides in the Violaceae. In: *Advances in Botanical Research V. 76*, Ed. Craik D.J. 2015, 76, Chapter 2: 15-49

Jafari MH, Zarrabi M, Ghadam P, Keshavarzi M. Optimization of cyclotide like peptide extraction methods and characterization of these peptides from *Viola tricolor*. *Health Biotechnology and Biopharma* 2017, 1(2): 25-38

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Hellinger R, Koehlbach J, Soltis DE, Carpenter EJ, Wong GK-S, Gruber CW. Peptidomics of circular cysteine-rich plant peptides: analysis of the diversity of cyclotides from *Viola tricolor* by transcriptome and proteome mining. *Journal of proteome research* 2015, 14: 4851-4862

Khoshkam Z, Zarrabi M, Sepehrizade Z, Keshavarzi M. The study of antimicrobial activities of partially purified cyclotide content and crude extracts from *Viola tricolor*. *J Med Bacteriol* 2016, 1(2): 29-35

Kirichenko TV, Sobenin IA, Markina YV, Gerasimova EV, Grechko AV, Karhirsich DA, et al. Clinical effectiveness of a combination of black elder berries, violet herb and Calendula flowers in chronic obstructive pulmonary disease: the results of a double-blind placebo-controlled study. *Biology* 2020, 9, 83, in press, doi 10.3390/biology9040083

Mousavi SH, Naghizade B, Pourgonabadi S, Ghorbani A. Protective effect of *Viola tricolor* and *Viola odorata* extracts on serum/glucose deprivation-induced neurotoxicity: role of reactive oxygen species. *Avicenna J Phytomed* 2016, 6(4): 434-441

Piana M, Silva MA, Trevisan G, de Brum TF, Silva CR, Boligon AA, et al. Antiinflammatory effects of *Viola tricolor* gel in a model of sunburn in rats and gel stability study. *Journal of Ethnopharmacology* 2013a, 150: 458-465

Piana M, Zadra M, de Brum TF, Boligon AA, Gonçalves AFK, da Cruz RC, et al. Analysis of rutin in the extract and gel of *Viola tricolor*. *Journal of Chromatographic Science* 2013b, 51: 406-411

Rahimi VB, Askari VR, Hosseini M, Yousefsani BS, Sadeghnia HR. Anticonvulsant activity of *Viola tricolor* against seizures induced by pentylenetetrazol and maximal electroshock in mice. *Iran J Med Sci* 2019, 44(3): 220-226

Sadeghnia HR, Hesari TG, Mortazavian SM, Mousavi SH, Tayarani-Narajan Z, Ghorbani A. *Viola tricolor* induces apoptosis in Cancer cells and exhibits antiangiogenic activity on chicken chorioallantoic membrane. *BioMed Research International* 2014. Article ID 625792, 8 pages, in press, doi <http://dx.doi.org/10.1155/2014/625792>

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Schumacher JB, Piana M, Forbrig Foeder AL, Gindri AL, da Cruz RC, de Freitas RB, et al. Dosamento de flavonoides em extracto liofilizado da *Viola tricolor* L. *Revista Contexto & Saude, Ijuí* 2011, 10 (20): 907-910 [Port.]

Tang J, Wang CK, Pan X, Yan X, Zeng G, Xu W, et al. Isolation and characterization of cytotoxic cyclotides from *Viola tricolor*. *Peptides* 2010, 31: 1434-1440

Wianowska D, Dawidowicz AL, Bernacik K, Typek R. Determining the true content of quercetin and its derivatives in plants employing SSDM and LC-MS analysis. *Eur Food Res Technol* 2017, 243: 27-40

b) References that justify the need for the revision of the monograph:

None

Rapporteur's proposal on revision

- ☐ Revision needed, i.e. new data/findings of relevance for the content of the monograph
- ☒ No revision needed, i.e. no new data/findings of relevance for the content of the monograph

HMPC decision on revision

- ☐ Revision needed, i.e. new data/findings of relevance for the content of the monograph
- ☒ No revision needed, i.e. no new data/findings of relevance for the content of the monograph

The HMPC agreed not to revise the monograph, assessment report and list of references on *Viola tricolor* L. and/or subspecies *Viola arvensis* Murray (Gaud) and *Viola vulgaris* Koch (Oborny), herba cum flore by consensus.