Affron (R) and increase in positive mood: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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Affron® and increase in positive mood: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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Abstract

Following an application from Pharmactive Biotech Products, S.L. submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Spain, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to affron® and contributes to maintain a healthy mood. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The food proposed by the applicant as the subject of the health claim is affron®, an aqueous saffron extract with a content of the sum of crocins and safranal typically between 3.5% and 3.9%. The Panel notes that affron® is sufficiently characterised. The claimed effect proposed by the applicant is 'contributes to maintain a healthy mood'. The Panel notes that increase in positive mood is a beneficial physiological effect for individuals with low mood or anxiety. One human intervention study showed that consumption of affron® at a dose of 28 mg/day for 4 weeks improves mood in a population of adults with low mood. However, the results have not been replicated in other studies. The information supplied by the applicant did not provide evidence for a plausible mechanism by which affron® could exert the claimed effect. The Panel concludes that the evidence is insufficient to establish a cause and effect relationship between the consumption of affron® and increase in positive mood.

Keywords: affron®, saffron, crocins, safranal, mood, anxiety, health claim

Requestor: Competent Authority of Spain following an application by Pharmactive Biotech Products, SL

Question number: EFSA-Q-2020-00617

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Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence or include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3). According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: affron® and increase in positive mood.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of affron®, a positive assessment of its safety, nor a decision on whether affron® is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food for which the health claim is made is 'Aqueous saffron extract, affron®, with the sum of crocins and safranal concentration > 3.5% and dextrin as inert carrier'.

Health relationship as claimed by the applicant

According to the applicant, the health effect is stated as 'improves mood by reducing the negative traits of depressive and anxiety feelings without side-effects'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant states that: 'Saffron seems to have a multi-target effect due to two specific metabolites: safranal and crocetin (the assimilable carotenoid form). Saffron is an antagonist of the 5-HT2c receptors, as some of the first-line antidepressants. Maybe it has some MAO inhibitor effect. Saffron has antioxidant and anti-inflammatory effects beside its impact on the activity of the HPA axis. Furthermore, saffron has neuroprotective and neurogenesis enhancing effects via the induction of BDNF expression and some results indicate that it also acts as a dopamine, noradrenaline and serotonin reuptake inhibitor'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'affron® contributes to maintain a healthy mood by reducing the negative traits of depressive and anxiety feelings'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is 'general healthy population, 12 years old or older with feelings of low mood or anxiety'. The quantity of 28 mg/day (oral consumption) is recommended.

Data provided by the applicant

The health claim application on 'affron® contributes to maintain a healthy mood' pursuant to Article 13.5 of Regulation (EC) No 1924/2006, was presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims (EFSA NDA Panel, 2016).

As outlined in the General guidance for stakeholders on health claim applications, it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claim applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

The scientific requirements for health claims related to functions of the nervous system, including psychological functions are outlined in a specific EFSA guidance (EFSA NDA Panel, 2012).

The application does not contain data claimed as proprietary or data claimed as confidential.

3. Assessment

The approach used by the NDA Panel for the evaluation of health claims is explained in the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016). In assessing each specific food/health relationship, which forms the basis of a health claim the NDA Panel considers the following key questions:

i) the food/constituent is defined and characterised;
ii) the claimed effect is based on the essentiality of a nutrient; OR the claimed effect is defined and is a beneficial physiological effect for the target population and can be measured *in vivo* in humans;
iii) a cause and effect relationship is established between the consumption of the food/constituent and the claimed effect (for the target group under the proposed conditions of use).

Each of these three questions needs to be assessed by the NDA Panel with a favourable outcome for a claim to be substantiated. In addition, an unfavourable outcome of the assessment of questions (i) and/or (ii) precludes the scientific assessment of question (iii).

3.1. Characterisation of the food/constituent

The food/constituent proposed by the applicant as the subject of the health claim is 'aqueous saffron extract, affron®, with the sum of crocins and safranal concentration ≥ 3.5% and dextrin as inert carrier'.

affron® is a dry powdered extract of saffron, dried *Crocus sativus* stigmas with a content of the sum of crocins and safranal that is ≥ 3.5%. In the analytical results provided in relation to the batch-to-batch variability, the maximum content was 3.9%. Typically, crocins constitute 99.5% and safranal 0.5% of this combination. As described in the studies provided to support the claim, the saffron is cultivated in Alborea (Albacete, Spain).

Crocins (or crocetin esters) are non-provitaminic carotenoids, in which the carboxylic functions of crocetin are esterified by glucose, gentiobiose or neapolitanose (Ahrazem et al., 2015). The major crocetin ester in saffron is *trans*-crocetin di-(β-D-gentiobiosyl) ester (CAS 42553-65-1), which amounts to about one half of total crocins.

Safranal is a monocyclic monoterpene with a melting point < 25°C and a boiling point of 70°C. This compound is the major compound responsible for the typical saffron aroma.

The content of crocins and safranal in affron® has been measured by the applicant using spectrophotometry without prior separation. Spectrophotometric measurements at the maximum absorbance wavelength are used to determine the concentration of safranal and crocin. In addition,

high-performance liquid chromatography in reverse phase mode (RP-HPLC) is used. It enables the correct quantification of safranal and crocins independently.

affron® can be included in different foods and food supplements. In the studies submitted it was used as coated tablets containing 11 or 14 mg of the standardised saffron extract in which dextrin was used as carrier.

An overview of the manufacturing process and information regarding batch-to-batch variability and stability of batches was provided.

The Panel considers that affron®, an aqueous saffron extract with a content of the sum of crocins and safranal typically between 3.5% and 3.9%, which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'improves mood by reducing the negative traits of depressive and anxiety feelings'. The proposed target population is 'general healthy population, 12 years old or older with feelings of low mood or anxiety'.

Affect encompasses defined states or traits such as positive (characterised by, e.g. enthusiasm and calmness) or negative (characterised by, e.g. confusion, feeling depressed, fatigue, tension and anxiety) mood. Enhancement of mood/affect (i.e. the increase, maintenance or reduced loss of one or more positive affect traits; the decrease in one or more negative affect traits) is a beneficial physiological effect for subjects wishing to improve their mood.

The scientific evidence for the substantiation of health claims related to the enhancement of mood/affect in one or more of its traits can be obtained from human intervention studies showing an effect on self-reported measures of the specific trait(s) by using comprehensive assessment tools (e.g. comprehensive self-rating adjective checklists or visual analogue mood scales) and/or specific, valid and reliable tests for the particular trait(s) of mood/affect which is (are) the subject of the claim.

Evidence for a sustained effect with repeated consumption of the food/constituent should be provided. Evidence for an effect on the incidence of clinically diagnosed depression by using valid clinical diagnostic tools could also be used for the scientific substantiation of a claim on the enhancement of mood (EFSA NDA Panel, 2012).

Evidence that the comprehensive or specific psychometric tests used for the subjective assessment of mood/affect endpoints are appropriate for the study population should be provided. When experimental mood-induction techniques are used in human intervention studies, evidence/a rationale for the validity of such experimental models should also be provided, and will be considered on a case-by-case basis as supportive evidence for the scientific substantiation of these claims.

With respect to the study population, the rationale for extrapolation of results obtained in patients with a clinically diagnosed affective disorder (e.g. depression) to the target population for the claim (e.g. subjects without the disorder) should be provided, and will be considered on a case-by-case basis (e.g. evidence on whether the mechanism by which the food constituent may exert the claimed effect on enhancement of mood in subjects with the disease is also relevant for subjects without the disease). Where appropriate, the confounding role of medication should be considered (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect) (EFSA NDA Panel, 2012).

The Panel considers that increase in positive mood is a beneficial physiological effect for individuals with low mood or anxiety.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed with the following keywords: saffron, affron®, extract, depression, anxiety, mood, antidepressant. The search was restricted to randomised, parallel, double-blind clinical trials carried out with participants ≥ 12 years old and to the specific affron® product.

Three human intervention studies considered as relevant by the applicant were retrieved by the search.

Lopresti et al. (2019) studied the effect of affron® 28 mg administrated for 8 weeks on mood in a group of patients with depression in a randomised, parallel, two-arm, double-blind, placebo-controlled study.

Included were physically healthy people with diagnosed depression, aged 18–65 years, currently taking a stable dose (at least 8 weeks) of a single pharmaceutical antidepressant. Despite antidepressant treatment, participants continued to suffer from mild-to-moderate depressive symptoms as assessed by a score greater than 6 on the Montgomery–Åsberg Depression Rating Scale (MADRS) (9 items). They could participate in psychological therapy if treatment started at least eight weeks prior to the enrolment to the study. Non-included were patients with psychiatric disorder other than mild-to-moderate depression or anxiety, self-harm behaviours and/or reported serious suicidal ideation.

The applicant was asked to explain how the results of the study with patients diagnosed with depression with mild-to-moderate depressive symptoms taking antidepressants can be extrapolated to the target population for the claim defined as a general healthy population with feelings of low mood. In reply, the applicant stated that the results from this study cannot be used to extrapolate the effects of affron® in people not taking antidepressant medication.

The Panel considers that the results of this study conducted in patients with pharmacologically treated depression cannot be extrapolated to the target population of the claim, i.e. general healthy population, 12 years old or older with feelings of low mood or anxiety. Therefore, no conclusions can be drawn from this study for the scientific substantiation of the claim.

Kell et al. (2017) in a randomised, parallel, three-arm, double-blind, placebo-controlled, single centre study compared the effect of two doses of affron® (22 and 28 mg/day) to placebo (microcrystalline cellulose and calcium hydrogen phosphate) on mood in a group of 128 participants.

Eligible for the study were healthy adults recruited from the research centre's subject database and the public media with self-reported low mood. Participants were included if they scored > 10 and < 20 in the Beck's Depression Inventory (BDI), indicating mild depression. Not included were: patients with a diagnosed mood disorder, those who had positive results for depression on the Beck Depression Inventory (BDI > 20), individuals with increased anxiety specified as State-Trait Anxiety Inventory (STAI) scores more than one SD above the average of the general population on the state or the trait anxiety (≥ 46 points), and people with arterial hypertension, warfarin use and insomnia.

The participants were randomly assigned to one of three groups receiving affron® 22 or 28 mg/day, or placebo. Each participant took one coated tablet two times daily (in the morning and with the midday meal) for four weeks. The tablets containing 11 mg and 14 mg affron® and placebo were identical.

Mood was assessed two times: at baseline and at the end of the study. The following questionnaires were used: Profile of Mood States (POMS; the main outcome), The Positive and Negative Affect Schedule (PANAS); and Depression Anxiety Stress States (DASS-21). In addition, the quality of sleep was evaluated using the Pittsburgh Sleep Quality Index (PSQI).

POMS is a self-report scale used to assess transient, distinct mood states. It consists of 65 items. The answers are grouped into six subscales; five negative: tension, depression, anger, fatigue and confusion, and one positive: vigour. 5-point Likert scale is used for each item (from 0 to 4 points). Responses for six subscales (i.e. tension + depression + anger + fatigue + confusion – vigour) are combined to obtain a Total Mood Disturbance (TMD). High scores of TMD reflect a greater degree of mood disturbance. For each subscale and for TMD score changes from baseline to the end of the intervention are calculated (Heuchert and McNair, 2012).

PANAS comprises two self-reported 10-item mood scales. One of them measures positive affect and the other – negative affect. The replies reflect the feeling of the individual over the last week. The score changes from the beginning to the end of the intervention are assessed. The results are reported for two subscales (i.e. Positive Affect and Negative Affect) (Watson et al., 1988).

DASS-21 is a self-report scale used to evaluate the negative emotional state. It consists of three subscales (i.e. depression, anxiety and stress). All responses are placed on a 4-point Likert scale (from 0 to 3 points). The scores for each subscale are obtained by summing the scores for pertinent individual items. In the study, the changes of the score from baseline to the end of the intervention were assessed for each individual and each subscale (Lovibond and Lovibond, 1995).

The Panel notes that all the questionnaires are validated, and they are frequently used in the area of psychology and psychiatry.

The estimated sample size was based on the expected results in the POMS. A number of 93 participants was calculated to be needed to attain a power of 0.80 for two-tailed tests at an effect size of 15 (50 to 35 (SD = 20)) (31 per group) with an alpha level of 0.05. It was assumed by the authors of the study that the DASS-21 was more specific for assessing changes in Depression, Anxiety and Stress domains. Therefore, the required sample size was expected to be lower and thus the sample size calculated on the basis of the POMS was assumed to be also sufficient for the DASS-21.

For the statistical analyses, score changes from baseline to week four were calculated for each participant in each mood measure and assessed by one-way analysis of variance (ANOVA). Upon a request from EFSA, the applicant provided an analysis of absolute scores for each scale and subscale using an analysis of covariance (ANCOVA) with baseline scores as covariate. Post-hoc between group comparisons were adjusted for multiple pairwise comparisons using the Bonferroni correction.

In total, 128 adults (75 women, mean age 39 ± 13.8 years) were randomised: 43 in the 22 mg/day affron® group, 42 in the 28 mg/day affron® group and 43 in the placebo group. Seven participants were lost to follow-up (5 in the placebo group and one in each of the affron® groups).

The Panel noted some discrepancies between the data provided in the publication and in the Appendix to the study report. The applicant clarified that the discrepancies were related to the fact that the participants with missing values were not included in the summary statistics presented in the publication, contrary to the study report.

No significant differences in relation to any of the evaluated outcomes were observed at baseline between the study groups. In DASS-21 questionnaire, the average values at baseline were in the mild to moderate range (the mean scores were as follows: depression = 14.2, anxiety = 8.8, stress = 18.4, SD values not provided in the publication).

Both ANOVA and ANCOVA analyses indicated that affron® at a dose of 28 mg has a positive effect on symptoms related to stress and anxiety and decreases the symptoms of negative affect, whereas for the 22 mg dose no statistically significant effects were observed for any of the health outcomes. A statistical analysis of a formal dose-response relationship was not presented.

The results of the study (including both ANOVA and ANCOVA analyses) for the main outcome variables are summarised in Table 1.

Table 1: Results of Kell et al. (2017) study in relation to each scale and subscale of the POMS, PANAS and the DASS-21

	Mean change scores (± SD) in the 28 mg affron® group, the 22 mg affron® group and the placebo group	**ANOVA (28 mg affron® vs placebo)**	**ANCOVA (28 mg affron® vs placebo)**
POMS			
Total Mood Disturbance (TMD)	–30.8 ± 21.6 vs –18.4 ± 4 vs –5.4 ± 24.5	$p < 0.001$	Not provided
Depression	–8.4 ± 7.7 vs –4.3 ± 7.4 vs –1.3 ± 6.2	$p < 0.001$	$p < 0.002$
Confusion	–4.4 ± 4.1 vs –2.7 ± 4.1 vs –0.8 ± 3.4	$p < 0.001$	$p < 0.007$
Vigour	4.0 ± 5.5 vs 2.2 ± 5.7 vs –0.4 ± 6.6	$p = 0.007$	$p = 0.012$
Tension	–4.0 ± 4.7 vs –3.1 ± 4.7 vs –1.1 ± 4.8	$p = 0.025$	Not provided
Anger	–5.1 ± 5.1 vs –3.1 ± 6.9 vs –1.1 ± 4.8	$p = 0.037$	$p = 0.037$
Fatigue	–5.0 ± 5.3 vs –2.9 ± 4.6 vs –1.1 ± 6.3	$p = 0.009$	$p < 0.006$
PANAS			
Negative Affect	–6.6 ± 5.2 vs –3.9 ± 5.8 vs –2.4 ± 3.6	$p = 0.001$	$p = 0.005$
Positive Affect	4.3 ± 8.0 vs 3.1 ± 7.0 vs 0.9 ± 6.4	$p = 0.124$	p = NS
DASS-21			
Depression	–11.2 ± 7.5 vs –5.3 ± 8.5 vs –3.1 ± 5.9	$p < 0.001$	$p < 0.001$
Anxiety	–6.4 ± 6.9 vs –4.1 ± 5.7 vs –2.6 ± 5.6	$p < 0.010$	$p = 0.008$
Stress	–12.2 ± 7.7 vs –5.6 ± 7.6 vs –3.3 ± 8.0	$p < 0.001$	$p < 0.001$

The Panel notes that the ANCOVA results provided upon EFSA's request omit the analyses for the Tension and Total Mood Disturbance subscales of the POMS.

The Panel considers that this study shows an effect of affron® at a dose of 28 mg/day given for 4 weeks on mood in a population of adults with low mood.

Lopresti et al. (2018) report on a randomised, double-blind, parallel, two-arm, placebo-controlled study which assessed the effect of affron® on anxiety and depression in adolescents. Physically healthy children aged 12–16 years (n = 80), with mild-to-moderate anxiety or depressive symptoms, were included. The severity of symptoms was assessed using the Revised Child Anxiety and Depression Scale (RCADS), child and parent versions.

The RCADS-Child is a self-report questionnaire that assesses symptoms of depression and anxiety in children and adolescents. It consists of 47 items related to: separation anxiety disorder, social phobia, generalised anxiety disorder, panic disorder, obsessive compulsive disorder and low mood (major depressive disorder). The sum of the five anxiety subscales is called a Total Anxiety Scale and a sum of all five subscales related to anxiety and one related to depression – a Total Internalizing Scale. The RCADS-Child can be used to screen for and monitor symptoms of depression and anxiety and has been validated. The RCADS, parent version, assesses symptoms of anxiety and depression of children across the same subscales based on parents' reports. It can be used in conjunction with the RCADS-Child (Chorpita et al., 2000; de Ross et al., 2002).

The Panel notes that in relation to a claim on mood, the most relevant subscales of the RCADS are the subscales on separation anxiety, generalised anxiety and depression. The Total Anxiety Scale and the Total Internalizing Scale provide an overall summary score of the results of the individual scales and are also considered to provide important information in relation to a claim on mood.

The participants of the study were recruited through social media advertisements and television/ radio interviews with the investigators. They were included if a total or subscale raw score greater than the 60th percentile for respective age and gender was obtained on either the child or parent version of the questionnaire, based on established normative data. Non-inclusion criteria comprised current or 12 month history of any psychiatric disorder other than mild-to-moderate depression or anxiety disorder, or currently receiving, or planning to receive a mental health intervention, or having a total or subscale raw score on the RCADS (child or parent version) greater than the 90th percentile for their respective age and gender, based on established normative data, or engaging in self-harm behaviours and/or reporting thoughts of suicide. Upon a request from EFSA asking why the thresholds of 60th and 90th percentiles in RCADS were chosen, the applicant explained that the lower percentile (60th) was chosen to limit the likelihood of recruiting individuals with absent/normal affective symptoms for their respective age and gender and the higher percentile (90th) was chosen to limit the likelihood of recruiting individuals with a diagnosed affective disorder. In this relation, the applicant also submitted the Revised Children's Anxiety and Depression Scale User's Guide (Chorpita et al., 2021) based on an Australian sample of school-aged children from which the percentiles were taken.

The participants were randomised in blocks of 10 to two groups: affron® 28 mg/day for 8 weeks vs placebo (microcrystalline cellulose and calcium hydrogen phosphate). Both groups were advised to ingest two coated tablets, one tablet with the morning meal and one tablet with the midday meal. The products tested were identical in shape, colour and taste.

The RCADS-Child, that was the primary outcome of the study, was administered every 2 weeks (five times across the intervention). In parallel, the parents' version of the RCADS, a secondary outcome of the study, was gathered. Both scales were assessed by online questionnaires or phone interviews. Upon a request from EFSA whether these conditions could influence the results, the applicant replied that online and telephone interviews are commonly used as assessments for affective symptoms and disorders and there is no reason to believe that this should have compromised the validity of the findings. To support this statement, the applicant submitted several publications comparing online and paper psychometric instruments for the assessment of common mental health disorders (Carlbring et al., 2007; Holländare et al., 2010; van Ballegooijen et al., 2016; Cronly et al., 2018; Ballester et al., 2019).

Assuming a power of 80% and a type one error rate (alpha) of 5%, and using an expected effect size of 0.7, the number of individuals per group to detect an effect was estimated as 34.

The statistical analysis was performed using repeated-measures (RM) multivariate analysis of variance (MANOVA). EFSA requested the applicant to provide, as a sensitivity analysis, an analysis that included baseline as covariate. The applicant presented the results of an ANCOVA that, however, neglected the repeated measures design of the study. Therefore, this analysis was not further considered by the Panel.

In total, 80 participants were randomised (40 in the affron® group and 40 in the placebo group, mean age 14 years, 68% girls). Of this, 68 participants completed the study and 12 dropped-out (4 in the affron® group and 8 in the placebo group). Reasons for withdrawal were reported, including inconsistent tablet intake (n = 1), refusal to take tablets (n = 5), failure to complete questionnaires (n = 1), worsening mental health (n = 2) and commencement of psychological intervention (n = 2). One participant (from the placebo group) had self-reported nausea/headaches believed to be caused by tablet intake.

At baseline, there were no significant differences between the groups on any mood questionnaire scores or demographic variables.

In the RM-MANOVA analysis of the results of the RCADS-Child, a significant treatment per time interaction was observed (p = 0.049) in favour of affron®. The individual subscales for which significant treatment per time interactions were observed were separation anxiety (p = 0.003), social phobia (p = 0.023) and depression (p = 0.016), while no effects on generalised anxiety, panic and obsession/compulsion were found. The Total Anxiety Scale and the Total Internalizing Scale were not analysed, owing to problems with collinearity in the MANOVA analysis. When comparing differences between the placebo and the intervention group at individual time points for separation anxiety, social phobia and depression, using a t-test, no statistically significant differences were observed.

The Panel notes that, for RCADS-Child, a significant treatment per time interaction was observed in favour of affron® for separation anxiety and depression, but that comparisons at individual time points were not statistically significant, indicating the lack of a substantial effect of affron® on the outcomes. There was no effect on generalised anxiety. The Total Anxiety Scale and the Total Internalizing Scale were not assessed. The findings in the child questionnaire were not corroborated by the results of the parent questionnaire.

The Panel considers that no conclusions can be drawn from the results of this study with respect to whether the study shows or does not show an effect of affron® on mood.

The Panel notes that one human intervention study carried out in a population of adults with low mood shows that affron® in a dose of 28 mg/day given for 4 weeks improves mood.

Proposed mechanism of action

The applicant claims that saffron can have a potential positive influence on mood due to several mechanisms: anti-inflammatory effects, antioxidant effects, neuroprotective effects, hypothalamus–pituitary–adrenal (HPA) modulating effects. It is also claimed that saffron acts as a dopamine, noradrenaline and serotonin reuptake inhibitor.

The Panel notes that antioxidant and anti-inflammatory effects are discussed in the literature to have an effect on mood (Jones et al., 2020). However, to date there is no evidence for a causal relationship between inflammatory responses and mood.

The studies that were provided by the applicant and that investigated the effect of saffron extracts on inflammation and on brain-derived neurotrophic factor (BDNF) expression were related to outcomes not associated with mood (e.g. hunger, stroke, neuroprotective and neurogenesis effects). Therefore, the Panel considers that no conclusions can be drawn from the studies that investigated these outcomes on a claim on mood.

The human study by Fukui et al. (2011) that measured corticoid plasma concentrations after acute exposure to saffron odour was not further considered as it was a non-randomised study and investigated the effect of odour only.

In a study in rats, Ettehadi et al. (2013) measured brain dopamine, serotonin, norepinephrine and glutamate concentrations after intraperitoneal (i.p.) administration of aqueous extract of saffron stigma in doses of 5–250 mg/kg body weight (bw). The dopamine concentration in brain increased in a dose-dependent manner beginning from the dose of 50 mg/kg bw given i.p. The Panel notes that the doses of saffron extract used in this study were considerably higher than those proposed for the claim in humans.

Hosseinzadeh et al. (2004) performed forced swimming tests, a model of depressive-like behaviour that uses immobility time as a measure of despair, and open field tests, a model for anxiety-like behaviour, in mice 30 min after i.p. injection of aqueous saffron stigma extracts (80, 160 and 320 mg/kg bw), ethanolic saffron stigma extracts (200, 400, 800 mg/kg bw), pure safranal (0.15, 0.35 and 0.5 mg/kg bw) and crocin (50, 200 and 800 mg/kg bw). Their effects were compared with fluoxetine (10 mg/kg bw; selective serotonin reuptake inhibitor), imipramine (13 mg/kg bw; a tricyclic antidepressant, which inhibits the reuptake of certain neurotransmitters in the brain, including acetylcholine, dopamine, norepinephrine and serotonin) and saline (10 mL/kg bw). The two highest doses of the aqueous extracts and the lowest and highest dose of the ethanolic extracts of saffron, all doses of crocin and the highest dose of safranal showed a decrease in immobility time (as compared to the saline solution), as did fluoxetine and imipramine. The two highest doses of the aqueous extracts, all doses of the ethanolic extracts as well as the highest dose of safranal (but not crocin) increased swimming time, as did fluoxetine but not imipramine. Climbing time was reduced by both saffron extracts and safranal at the two lowest doses, similar to fluoxetine. Crocin and the highest dose of safranal (0.5 mg/kg) as well as imipramine significantly increased climbing time. In the open field test, total locomotion was significantly reduced by the aqueous extracts at the two higher doses as well as the highest does of safranal. There was no effect of the ethanolic extract, crocin and imipramine. No results are presented for fluoxetine. Based on these

results and the known mechanisms of action of the two antidepressant medicines, the authors suggest that crocin may act via the uptake inhibition of dopamine and norepinephrine, and safranal via serotonin.

In a study with similar design by Amin et al. (2015), the forced swimming test and the open field test were performed after an acute treatment of mice with crocin and crocetin (10, 20 and 40 mg/kg bw, i.p.), fluoxetine, desipiramine (both 10 mg/kg bw,i.p.) and saline. Mice were also subjected to the forced swim test, the tail suspension test, also a model of depressive-like behaviour, and the open field test following a subacute oral administration of these substances (crocin: 25, 50 and 100 mg/kg bw; crocetin 12.5, 25 and 50 mg/kg bw, and fluoxetine, desipiramine (both 10 mg/kg bw), given once daily for 21 days). Immobility time in mice was reduced, compared to the saline solution, by crocetin (starting from 20 mg/kg in acute test and at all doses in the subacute test) and the highest doses of crocin (40 mg/kg in acute test and 100 mg/kg in subacute test). Immobility time was also significantly reduced with fluoxetine and desipiramine. Results compared with the saline solution with respect to climbing time and swimming behaviour were not reported. All doses of crocetin, the highest dose of crocin as well as fluoxetine and desipiramine also reduced immobility time in the tail suspension test (subacute study only). There was no effect of any of the substances administered on the locomotor activity of animals in the open field test, neither in the acute nor in the subacute study.

The Panel notes that the doses of saffron extracts and the saffron ingredients used in these studies were many times higher than proposed for the claim in humans, that the observed effects were partly dependent on the dose administered and that the concentration of neurotransmitters in the brain was not measured, and that links of the specific components of the forced swimming test, the open field test and the tail suspension test with the effect exerted by specific neurotransmitters are hypothetical.

De Monte et al. (2014) investigated,in an *in vitro* study,the effect of crocin and safranal on human monoamine oxidases (hMAO-A and hMAO-B). They reported that crocin is a relatively weak inhibitor of the hMAOs, and safranal is not a hMAO inhibitor. The authors concluded that saffron is unlikely to exert an effect on the central nervous system through MAO inhibition by crocin and safranal.

Several studies submitted referred to the effect of saffron on HPA axis. Hooshmandi et al. (2011) investigated the effects of aqueous saffron extract and safranal on corticosterone plasma concentrations in rats under stress induced by electroshock. In animals which received intra-amygdala or i.p. saffron extract and safranal, an increase in plasma corticosterone following stress induction was not seen. Halataei et al. (2011) reported that in aqueous saffron extract- and crocin-treated mice the plasma corticosterone concentrations did not increase following stress-induced anorexia. The subcutaneous injection of crocin significantly decreased plasma levels of corticosterone in rats being exposed to chronic restraint stress (Ghadrdoost et al., 2011).

The Panel notes that these studies reported some effects of saffron extracts or constituents of saffron on cortisol/corticosterone concentrations under chronic stress conditions but their impact on long-term mood via the HPA axis remains unclear.

The Panel considers that the evidence provided by the applicant for the mechanisms by which saffron could exert an effect on mood is mostly speculative. The Panel considers that no reliable evidence has been provided by the applicant for a plausible mechanism by which saffron could exert the claimed effect *in vivo* in humans.

Weighing the evidence

In weighing the evidence, the Panel took into account that only one human intervention study showed that consumption of affron® at a dose of 28 mg/day for 4 weeks improved mood in a population of adults with low mood. However, the results have not been replicated in other studies. The information supplied by the applicant did not provide reliable evidence for a plausible mechanism by which affron® could exert the claimed effect.

The Panel concludes that the evidence is insufficient to establish a cause and effect relationship between the consumption of affron® and increase in positive mood.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food/constituent, affron®, aqueous saffron extract with a content of the sum of crocins and safranal typically between 3.5% and 3.9%, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'improves mood by reducing the negative traits of depressive and anxiety feelings'. The target population proposed by the applicant is 'general

healthy population, 12 years old or older with feelings of low mood or anxiety'. Increase in positive mood is a beneficial physiological effect for individuals with low mood or anxiety.
- The evidence is insufficient to establish a cause and effect relationship between the consumption of affron® and increase in positive mood.

Documentation as provided to EFSA

Health claim application on pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0499_ES). Submitted by Pharmactive Biotech Products, S.L., C/Faraday, 7. E-28049, Madrid, Spain.

Steps taken by EFSA

1) This application was received by EFSA on 17/09/2020.
2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
3) The scientific evaluation procedure started on 8/12/2020.
4) On 8/12/2020, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 21/12/2020 and was restarted on 3/01/2021, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
5) On 26/01/2021, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 18/02/2021 and was restarted on 2/03/2022, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
6) During its meeting on 26/05/2021, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to the consumption of and.

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Abbreviations

5-HT2c	5-hydroxytryptamine 2c
ANCOVA	analysis of covariance analysis of covariance
ANOVA	analysis of variance
BDI	Beck Depression Inventory
BDNF	brain-derived neurotrophic factor
bw	body weight
CAS	Chemical Abstracts Service
DASS-21	Depression Anxiety Stress States
hMAO	human mono amine oxidases
HPA	hypothalamus–pituitary–adrenal
HPLC	high-pressure liquid chromatography
i.p.	intraperitoneal
MADRS	Montgomery–Åsberg Depression Rating Scale
MANOVA	multivariate analysis of variance
NA	negative affect
NDA Panel	Panel on Nutrition, Novel Foods and Food Allergens

PA	positive affect
PANAS	Positive and Negative Affect Schedule
POMS	Profile of Mood States
PSQI	Pittsburgh Sleep Quality Index
RCADS	Revised Child Anxiety and Depression Scale
RM	repeated measures
SD	standard deviation
SSRI	selective serotonin reuptake inhibitor
STAI	State-Trait Anxiety Inventory
TCA	tricyclic antidepressant
TMD	Total Mood Disturbance