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affron® a novel saffron extract (*Crocus sativus L.*) improves mood in healthy adults over 4 weeks in a double-blind, parallel, randomized, placebo-controlled clinical trial

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ABSTRACT

Background: In recent years phytotherapy has been explored as a source for alternative treatments for mood disorders. One potential candidate is saffron (*Crocus sativus* L.), whose main bioactive components are crocins and safranal.

Objectives: The aim of this study was to investigate the efficacy of affron®, a standardised stigmas extract from *Crocus sativus* L. for improving mood, stress, anxiety and sleep quality in healthy adults.

Methods: In this 3 arm study, 128 participants self-reporting low mood but not diagnosed with depression, were given affron® at 28 mg/day, 22 mg/day, or a placebo treatment in a randomized, double-blind, placebo-controlled trial for 4 weeks. Mood was measured at baseline and at the end of the study, using the POMS (primary outcome measure) and PANAS questionnaires, and the DASS-21 scale. Sleep was monitored using Sleep Quality Index (PSQI).

Results: Analysis indicated a significant decrease in negative mood and symptoms related to stress and anxiety at a 28 mg/day dose (with a significant difference between 28 mg/day and placebo on the POMS Total Mood Disturbance scale, $p < 0.001$, $d = -1.10$), but no treatment effect at the 22 mg/day dose.

Limitations: The main weaknesses of this investigation were found in the self-reporting nature of both the screening and the testing.

Conclusions: affron® increased mood, reduced anxiety and managed stress without side effects, offering a natural alternative to standard treatments.

1. Introduction

Mental health disruptions are the leading cause of disability worldwide.[1] In Australia, for example, 45% of the population experienced a mental health condition in their lifetime (at least one of the selected mental disorders: anxiety, mood or substance use disorders), with one million adults suffering from depression, and over two million from anxiety.[2] The two conditions often co-exist; nearly half of those diagnosed with depression are also diagnosed with an anxiety disorder. Published prevalence figures for depression and anxiety only account for diagnosed cases, and true figures are certainly higher.

The number of affected people increases further when including those with subclinical depression, (i.e. those with low mood). These conditions share many symptoms and can be regarded as existing on a spectrum of disorder.

Depression, for example, is a medically defined pathology typically graded from profound down through severe and moderate to mild; whereas low mood describes a temporary emotional state characterized by symptoms usually associated with depression but less severe and/or prolonged,[3–5] with sub-clinical depression occupying a somewhat amorphous intermediate position.

While not considered a pathology, low mood is defined by many of

Abbreviations: POMS, Profile of Mood States; DASS-21, depression, anxiety and stress scale (21 question); PANAS, positive and negative affect scale; PSQI, Pittsburgh Sleep Quality Index

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the same symptoms used to define depression and sub-clinical depression, including sadness, crying, fatigue, pessimism, changes in appetite, changes in sleep patterns, and anhedonia. [6,3,4] Those who report such symptoms often struggle to cope with daily life yet lack the treatments available to those with a diagnosed disorder. Prescription medications are not only inappropriate in these instances, they are often ineffective.[7,8] The rates of remission tend to be low and the risk of relapse high.[9] Additionally, many find the adverse side effects of medications intolerable.[10] The search for alternative treatments has therefore become a high priority in the management of low mood.

Further impetus derives from evidence that both subclinical and chronic mild depression predispose to major clinical depression, [11,12] and low mood is likely also a risk factor.[13,14]

In recent years phytotherapy has been explored as a source for alternative treatments for mood disorders and depression. One potential candidate is saffron (*Crocus sativus* L.), whose main bioactive components, crocins and safranal, are responsible for the spice's aroma and characteristic red color.[15]

There is evidence that crocins act as reuptake inhibitors of dopamine and norepinephrine, while safranal acts primarily on serotonin reuptake.[16–19] The antioxidant properties of saffron derivates may also be relevant. Mood disorders are associated with elevated oxidative stress and a deficit of exogenous antioxidants, [20,21] affecting immune and inflammatory responses in a way, which may promote neurodegeneration.[22] There is good evidence that the antioxidants in saffron extracts protect against oxidative stress in the central nervous system, [23,24] constituting a second potential mechanism of therapeutic action.

The novel saffron extract affron® is characterized by HPLC–MS/ESI and standardised to safranal and crocins. The aim of this research was to measure the clinical efficacy of affron® for improving mood, reducing the symptoms of anxiety, stress, and improving vigour and sleep quality in healthy participants.

It was hypothesised that a change in mood scores (i.e., a decrease in negative mood scores and an increase in positives mood scores) on the POMS, PANAS, and DASS-21 over four weeks would be significantly greater in the active treatment groups than in the placebo group. It was also hypothesised that a change in these mood scores would be significantly greater in the active treatment groups than in the placebo.

All previous studies of saffron's effect on mood used 30 mg/day for either six or eight weeks, without examining the efficacy of lower dosage rates and shorter intervention periods. The benefits of exploring the minimal effective dose are not only therapeutic (to establish required dosage strength), they are also economic, especially for a spice as expensive as saffron. This study therefore investigated two lower dosage rates (22 mg/day, and 28 mg/day), and a shorter treatment time (four weeks).

2. Methods

2.1. Saffron extracts

A total of N = 8 batches of affron® samples (*Crocus sativus* L.) obtained from Pharmactive Biotech Products SL were employed for characterization. The samples were packaged in vacuum and stored in darkness at room temperature until analysis.

2.2. Reagents

Methanol and acetonitrile were purchased from Sharlau (Barcelona, Spain). All of the solvents were of HPLC degree, and the water used was bi-distilled and purified using a MilliQ Millipore system (Bedfore, MA).

Safranal and gallic acid reference substances, sodium carbonate and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (San Luis, USA), and *trans*-crocin-4 from Phytolab (Vestenbergsgreuth, Germany).

2.3. HPLC-PAD

High Performance Liquid Chromatography (HPLC) analysis of affron® samples was performed by means of an Agilent Technologies 1220 Infinity series system with photo-diode array detector (PAD), according to Caballero-Ortega et al. [25] The bioactive compounds safranal and crocins were quantified by means of safranal and *trans*-crocin-4 external calibration curves.

2.4. HPLC–MS

In order to confirm the identity of each peak, mass spectrometry (MS) was performed by means of Agilent series 1100 (Palo Alto, California, USA), coupled to mass-quadrupole detector (Hewlett-Packard, serie 1100 MSD), with electrospray ionization source (ESI), operated in positive and negative modes, according to Lech et al. [26]

2.5. Total phenolic compound content

Total phenolic compound content of affron® was performed by the colorimetric method of Singleton and Rossi, [27] using Folin-Ciocalteau reagent.

Data were expressed as mean value ± standard deviation of three independent measurements.

2.6. Clinical trial

The study was conducted in Brisbane, Australia, revised and approved by Queensland Clinical Trials Network Human Research and Ethics Committee, (Application number: HREC2014002). Australia, and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614001053617).

It was conducted in accordance with the Declaration of Helskinki, revised in 1989, and principles of the Australian Regulations on Medical Research involving Human Subjects (National Health and Medical Research Council; Australia).

2.7. Participants

A total of 128 healthy adults, aged 18–77 years were recruited from the CRO's subject database and the public media (Table 1). Participants were included for assessment if they were self-reporting low mood, were not diagnosed with depression or another mood disorder, were otherwise healthy (including BMI < 30). Participants were excluded if they had been diagnosed with a mood disorder or had tested positive for depression on the Beck Depression Inventory (BDI > 20). A minimum BDI score was not set, but only those reporting with low mood enrolled in the study.

Key exclusion criteria included: received and/or prescribed Coumadin (Warfarin), Heparin, Dalteparin, Enoxaparin or other anticoagulation therapy; diagnosed with hypertension and receiving and/or prescribed antihypertensive medications, diagnosed with severe renal and/or hepatic insufficiency; had a history of chronic alcohol and/or drug abuse; had participated in any other clinical trial during last 30 days; were currently participating in another clinical trial; diagnosed with a mood disorder (major depressive disorder (MDD), bipolar disorder or substance-induced disorder); had tested positive for moderate to severe depression on the Beck Depression Inventory; suffered from insomnia or had night-shift employment and were unable to have a normal night's sleep; suffered severe Pre-Menstrual Syndrome (PMS) with mood or pain that would change during the study period; suffered from any neurological disorder such as multiple sclerosis; were currently taking supplements (nutrients, including herbs) that would impact mood (St John's Wort, Tryptophan, SAM-E, 5-hydroyxtryptophan, Melatonin, GABA); were taking a saffron supplement or could not exclude foods containing saffron or the use of saffron in cooking.

Table 1
Participant Demographics at Baseline. Active treatment groups and placebo group evenly matched at baseline in all demographics, with no significant differences between groups.

Demographics		Treatment group		
	Total (N = 121)	28 mg/day (n = 41)	22 mg/day (n = 42)	Placebo (n = 38)
Age				
Mean (SD)	39.1 (13.77)	40.4 (12.71)	36.7 (14.59)	40.38 (13.97)
Range	18–77	21–68	18–77	23–68
Gender (Number, %)				
Female	75 (62.0%)	26 (63.4%)	26 (61.9%)	23 (60.5%)
Male	46 (38.0%)	15 (36.6%)	16 (38.1%)	15 (39.5%)
Status (Number, %)				
Partner	74 (61.2%)	25 (61.0%)	27 (64.3%)	22 (57.9%)
Single	47 (38.8%)	16 (39.0%)	15 (35.7%)	16 (42.1%)
Working (Number, %)				
Employed / student	103 (85.1%)	34 (82.9%)	37 (88.1%)	32 (84.2%)
Unemployed / retired	18 (14.9%)	7 (17.1%)	5 (11.9%)	6 (15.8%)
Weight				
Mean kg (SD)	76.34 (17.22)	75.89 (16.48)	77.54 (18.20)	75.56 (17.39)
BMI				
Mean (SD)	26.42 (6.33)	26.74 (5.90)	27.01 (7.91)	25.38 (4.77)
Smoking (Number, %)				
Yes	17 (14.0%)	8 (19.5%)	6 (14.3%)	3 (7.9%)
No	104 (86.0%)	33 (80.5%)	36 (85.7%)	35 (92.1%)
Alcohol (Number, %)				
3 or less per week	44 (36.4%)	14 (36.1%)	12 (28.6%)	18 (47.4%)
Over 3 per week	77 (63.6%)	27 (65.9%)	30 (71.4%)	20 (52.6%)

Note: No significant differences in demographics between treatments at baseline ($p > 0.05$, two-tailed).

2.8. Design and intervention

The study was a parallel, double-blind placebo-controlled design. The participants, self-reporting low mood but not diagnosed with depression, were included and randomly assigned to groups receiving the saffron extract (affron®, 22 or 28 mg/day), or placebo for 4 weeks.

The active treatment was a TGA listed coated tablet containing either 11 mg or 14 mg of standardised saffron extract (affron®), derived from the stigmas of Crocus sativus L. and standardised to contain > 3.5% Lepticrosalides® a measure of bioactive compounds present in saffron, including safranal and crocin The placebo tablet contained the same excipients as the active tablet (microcrystalline cellulose and calcium hydrogen phosphate). The active and placebo tablets were matched for size shape and coating color. Treatment containers were randomised using Random Allocation Software version 1.0, and labelled with a code. Participants were allocated a corresponding code (e.g., participant 15 received container 15). The randomisation code was maintained by the sponsor to keep the investigators blind and to facilitate code breaking in the case of adverse events. Placebo tablets were identical in appearance to active tablets and contained carrot extract instead of affron® (data not shown). The investigator was informed of treatment group allocation post-trial for statistical analyses.

2.9. Outcomes

Mood was measured at baseline and at the end of the study, using the following validated questionnaires: Profile of Mood States (POMS; primary outcome), The Positive and Negative Affect Schedule, (PANAS) and Depression Anxiety Stress States (DASS-21). Sleep was monitored using Pittsburgh Sleep Quality Index (PSQI).

POMS, [28] consists of 65 items, adjectives describing an emotion rated on a five-point scale, where 0 = not at all; 1 = a little; 2 = moderately; 3 = quite a lot; and 4 = extremely (except the items relaxed and efficient, reverse scored). Participants were asked how they felt at that moment, and answers were grouped into six subscales; five negative: Tension (ranged from −36 to 36), Depression (ranged from −60 to 60), Anger (ranged from −48 to 48), Fatigue (ranged from −28 to 28) and Confusion (ranged from −28 to 28) and one positive (Vigour; ranged from −32 to 32). A Total Mood Disturbance (TMD) score was calculated for each participant (Tension + Depression + Anger + Fatigue + Confusion − Vigour) to give an overview of mood state. Change scores from baseline to the end of the study were calculated for each subscale [29] and for TMD, possible scores ranged from −232 to 200.

PANAS, [30] consists of 20 items; 10 positive and 10 negative words. Scoring was on a five-point scale, where 1 = very slightly or not at all; 2 = a little; 3 = moderately; 4 = quite a bit; and 5 = extremely. Participants were asked how they felt over the previous week and answers were grouped into two subscales (Positive Affect, PA; and Negative Affect, NA). The change scores across time (from baseline to the end of the study), for both PA and NA were ranged from −40 to 40.

DASS-21, [31] is designed to measure stress, anxiety, and depression. It consists of 21 self-report items in the form of statements; seven forming the subscale of Depression, seven forming the subscale of Anxiety and seven forming the subscale of Stress. Participants were asked how they felt over the past week and scored each item from 0 to 3, where 0 = never; 1 = sometimes; 2 = often; and 3 = almost always. The variance of the scores across time (from baseline to the end of the study) was calculated for each subscale and each participant.

PSQI, [32] is designed to measure sleep quality using 19 self-rated questions and five questions rated by a person who had close relationship with the participant.

2.10. Procedure

Participants were provided with information about the product at the baseline interview, where they gave written informed consent and were advised that they could withdraw at any time. Medical details were then recorded, exclusion criteria checked and demographic data was gathered.

Participants completed the POMS, PANAS, DASS-21, and PSQI at the baseline interview and at the week four interview in the clinic of the investigator (Brisbane). The battery of tests took approximately 30 min per participant.

Once assigned to groups, participants were allocated either 28 mg/day or 22 mg/day of active treatment, or placebo. Each participant was instructed to take two tablets daily for four weeks, one tablet with the morning meal and one tablet with the midday meal. Product containers were returned at the week four interview, and any remaining tablets were recorded. Participants were asked at week 2 and the final interview if there had been any changes to their lifestyle, weight, or if they had noticed any adverse symptoms since starting treatment.

2.11. Statistical analyses

A priori power analyses conducted using G*Power version 3.1.9.2 [33] determined a sample size of 93 was required to attain a power of 0.80 for two-tailed tests detecting a large effect size (31 per group). To allow for exclusions and a 30% drop out the aim was to screen 140 participants. Therefore the final sample size of 121 was adequate for the a priori power requirement. In order to control for family wise a conservative Bonferoni corrections was applied to all the data.

Clinical study analyses were completed using IBM Statistical Package for Social Sciences (SPSS) version 23 at an alpha level of 0.05.

Change scores from baseline to week four were calculated for each participant in each mood measure, to reduce within group variance. [29] The group means of these change scores were used to assess the statistical difference between groups by one-way independent ANOVA. Gabriel's pairwise test procedure was used for post-hoc analyses since this was a three arm study with marginally different group sizes (28 mg/day, $n = 41$; 22 mg/day, $n = 42$; placebo, $n = 38$). Gabriel's post hoc test was chosen since it was designed to cope with slightly unequal group sizes.

3. Results

3.1. Chemical analysis

The HPLC-PAD/MS analysis of affron® identified six crocin isomers, together with picrocrocin, safranal and one kaempferol diglucoside. These results are in a good agreement with those obtained by Lech et al. [26]

As can be observed in Table 3, affron® samples showed a minimum content of safranal of 0.03%, whereas total crocin content was over 3.99% on average and a total phenolic compound content of 1.41%.

The sum of the bioactive components safranal and crocin isomers analysed by HPLC, which are also responsible for the main organoleptic properties, [15] herein is referred as Lepticrosalides®. [34] The proposed expression of results by HPLC is more objective and is expected to be more reproducible from laboratory to laboratory than the traditional ISO 3632 methodology. [35]

3.2. Clinical study results

After screening 137 potential participants for exclusion criteria, 128 healthy adults aged 18–77 years were randomised into three groups (Table 1). Seven participants were lost to follow-up, leaving 121 participants at completion, allocated to three groups; 28 mg/day, 22 mg/day, and placebo (Fig. 1). The mean age of participants was 39 years. The total sample was 62% female and 38% male. The mean weight at baseline was 76.3 kg. The mean BMI at baseline was 26. The majority of participants had partners, were working or studying, were non-smokers, consumed over 3 drinks per week, and reported undertaking regular exercise.

No significant differences between groups were observed at baseline in any of the outcomes, and low mood scores for the average participant at baseline were in the mild to moderate range, according to the DASS (average scores; depression = 14.2, anxiety = 8.8, stress = 18.4).

Change scores from baseline to week four were calculated for each participant in each mood measure for use in analyses. This reduced within-group variability that results from individual response specificity. The group means of the change scores were used to assess the statistical difference between groups by one-way independent ANOVA with a Gabriel's post hoc comparison test. Table 2 shows the mean change scores for each scale and subscale.

Table 2
Mean Change Scores (SD) for Each Scale and Subscale.

	Treatment group		
Baseline Mean (SD)	28 mg/day	22 mg/day	Placebo
POMS Total Mood Disturbance (-32 to 200)	40.2 (38.3)	31.5 (36.2)	38.5 (27.8)
PANAS Positive Affect Score Baseline (10–50)	27.8 (9.3)	27.3 (7.4)	29.8 (10.4)
PANAS Negative Affect Score Baseline (10–50)	20.8 (8.9)	19.3 (6.9)	18.6 (7.1)
DASS21 Depression Score Baseline (0–21)	6.7 (5.8)	6.3 (5.6)	6.4 (6.3)
Change scores per Instrument			
POMS			
Tension	−4.00 (4.65)	−3.10 (4.74)	−1.06 (4.80)
Depression	−8.43 (7.66)	−4.28 (7.43)	−1.33 (6.22)
Anger	−5.05 (5.05)	−3.10 (6.91)	−1.14 (4.80)
Fatigue	−5.00 (5.34)	−2.85 (4.58)	−1.11 (6.31)
Confusion	−4.35 (4.07)	−2.65 (4.07)	−0.83 (3.39)
Vigour	4.00 (5.46)	2.23 (5.71)	−0.39 (6.58)
Total Mood Disturbance	−30.83 (21.56)	−18.36 (27.62)	−5.37 (24.52)
PANAS			
Positive Affect	4.32 (8.04)	3.13 (7.00)	0.91 (6.43)
Negative Affect	−6.63 (5.24)	−3.92 (5.84)	−2.40 (3.65)
DASS			
Depression	−11.22 (7.48)	−5.29 (8.53)	−3.05 (5.86)
Anxiety	−6.44 (6.94)	−4.05 (5.65)	−2.63 (5.58)
Stress	−12.24 (7.74)	−5.62 (7.63)	−3.26 (8.03)
PSQI Global Score	−2.69 (2.61)	−2.27 (3.04)	−0.82 (2.77)

3.2.1. POMS

As shown in Fig. 2A, all subscales demonstrated a significant improvement as measured by the change scores by week 4 for the group treated with 28 mg/day of affron® compared to the rest of groups studied. In order to control for family wise error a conservative Bonferoni corrections was applied where the new alpha was set at ($p = 0.038$).

For the POMS Tension, Depression, and Confusion subscales, a significant treatment over time effect was observed (Tension, $F(2,113) = 3.82$; $p = 0.025$; Depression, $F(2,113) = 9.46$, $p < 0.001$, ω (effect size) = 0.36; Confusion, $F(2,113) = 7.81$, $p = 0.001$, $\omega = 0.32$). Gabriel's post hoc test revealed a significant decrease in the above subscales, in the 28 mg/day group compared to the placebo group, (Depression, $p < 0.001$, $d = -1.02$; and Confusion, $p < 0.001$, $d = -0.94$; respectively, indicating a large effect size according to Cohen's conventions).

A significant improvement for the POMS Fatigue subscale was also observed ($F(2.113) = 4.92$, $p = 0.009$, $\omega = 0.25$). Gabriel's post hoc test revealed a significant decrease in fatigue in the group treated with 28 mg/day of affron® in comparison with the placebo group, ($p = 0.007$, $d = -0.67$; a medium effect size according to Cohen's conventions).

Furthermore, there was a significant positive improvement for the POMS Vigour subscale ($F(2.112) = 5.25$, $p = 0.007$, $\omega = 0.26$). Gabriel's post hoc test revealed a significant increase in vigour in the 28 mg/day group compared to the placebo group, ($p = 0.005$, $d = 0.73$; a medium effect size according to Cohen's conventions).

Overall, there was a significant treatment effect for the POMS Total Mood Disturbance (TMD) scale ($F(2.111) = 9.94$, $p < 0.001$, $\omega = 0.37$). Gabriel's post hoc test revealed a significant decrease in TMD in the group that consumed 28 mg/day of affron® compared to the placebo group, ($p < 0.001$, $d = -1.10$; a large effect size according to Cohen's conventions), (Fig. 2B).

3.2.2. PANAS

Analysis revealed no significant between-group treatment effect on the change scores during the study for Positive Affect ($F(2.111) = 2.13$, $p = 0.124$), but it did show a significant improvement regarding the Negative Affect ($F(2.111) = 6.97$, $p = 0.001$, $\omega = 0.31$). Gabriel's post hoc test revealed a significant decrease in negative affect in the group treated with 28 mg/day of affron® compared to the placebo group, ($p = 0.001$, $d = -0.42$) (Fig. 3).

3.2.3. DASS-21

There was a significant treatment effect on the change scores for the DASS Depression subscale, $F(2.118) = 12.96$, $p < 0.001$, $\omega = 0.41$ (a large effect size). Gabriel's post hoc test revealed that a decrease in depression in the 28 mg/day group was significantly greater than in the 22 mg/day group and the placebo group, ($p < 0.001$, $d = -1.22$; $p = 0.001$, $d = -0.74$) respectively. The 22 mg/day group did not significantly differ in depression from the placebo group ($p = 0$ 0.449).

There was no significant treatment effect on the change scores for the DASS Anxiety subscale, $F(2.118) = 4.33$, $p = 0.01$, $\omega = 0.23$. Gabriel's

Table 3
HPLC analysis (%, dry weight) of safranal and crocin isomers and spectrophotometric quantitative analysis (%, dry weight) of total phenolic compound content in affron® samples (N = 8).

Analyte	Mean ± SD (%)	Range (%)	Proportion (%)				
			P10	P25	P50	P75	P90
safranal	0.04 ± 0.01	0.03–0.07	0.03	0.03	0.04	0.05	0.07
trans-crocin-4	2.88 ± 0.59	2.06–3.81	2.31	2.49	2.77	3.29	3.59
Total crocins	5.33 ± 0.95	3.99–6.86	4.33	4.81	5.18	5.79	6.60
TPCC[a]	1.41 ± 0.20	1.10 ± 1.71	1.17	1.28	1.45	1.53	1.63

[a] Total phenolic compound content by Folin-Ciocalteu reagent.

post hoc test revealed that a decrease in anxiety in the 28 mg/day group was significantly greater than in the placebo group, $p = 0.010$, $d = -0.65$. The 22 mg/day group did not significantly differ in anxiety from the 28 mg/day group ($p = 0.149$), or the placebo group ($p = 0.625$).

There was a significant treatment effect on the change scores for the DASS Stress subscale, $F(2.118) = 14.29$, $p < 0.001$, $\omega = 0.42$. Gabriel's post hoc test revealed that a decrease in stress in the 28 mg/day group was significantly greater than in the placebo group and 22 mg/day group, ($p < 0.001$, $d = -1.14$; $p = 0.001$, $d = -0.86$) respectively. The 22 mg/day group did not significantly differ in stress from the placebo group ($p = 0.445$). (Fig. 4).

3.2.4. PSQI

The effect of the saffron extract on sleep quality was analysed by the

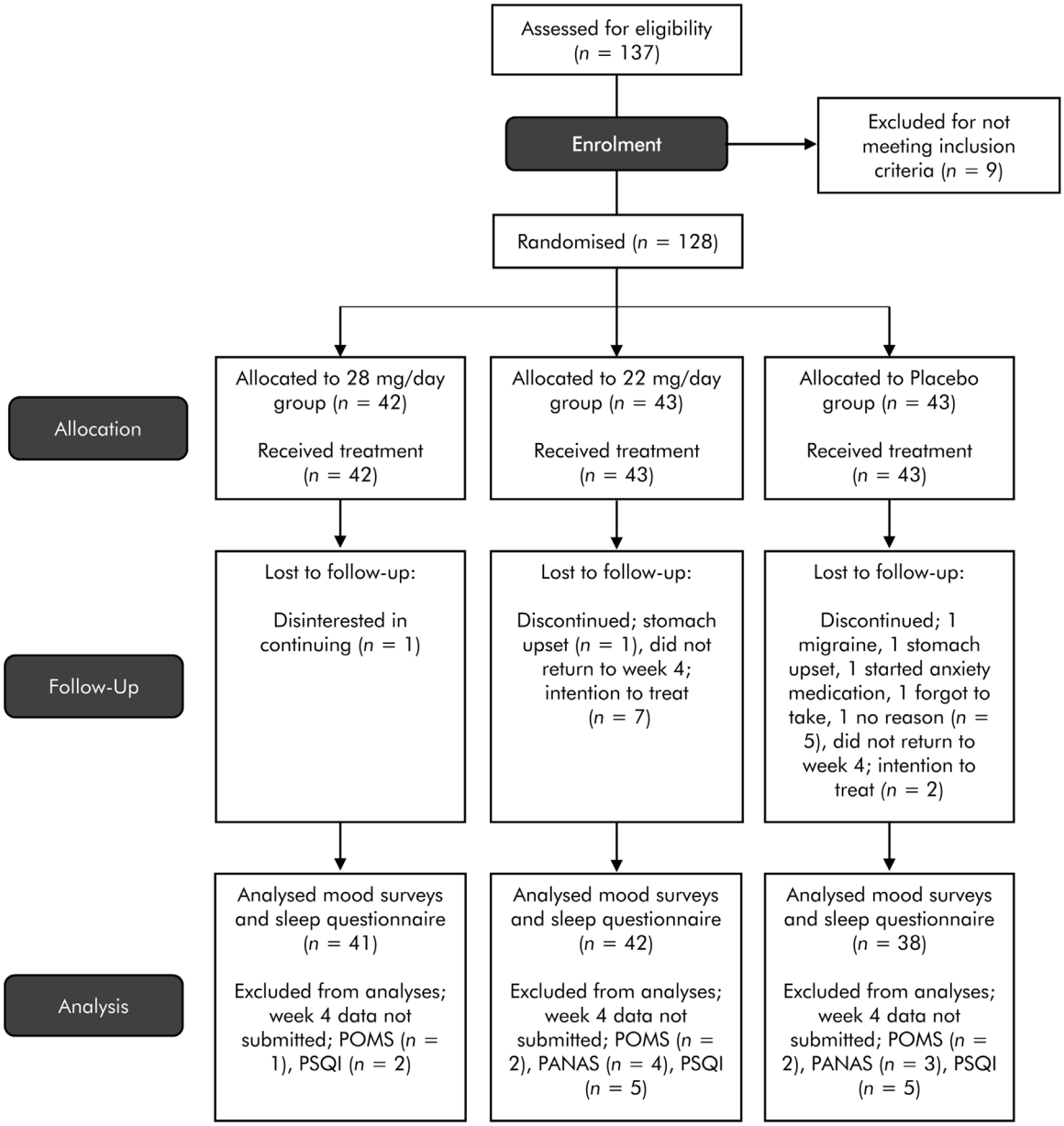

Fig. 1. Participant Flow Chart.

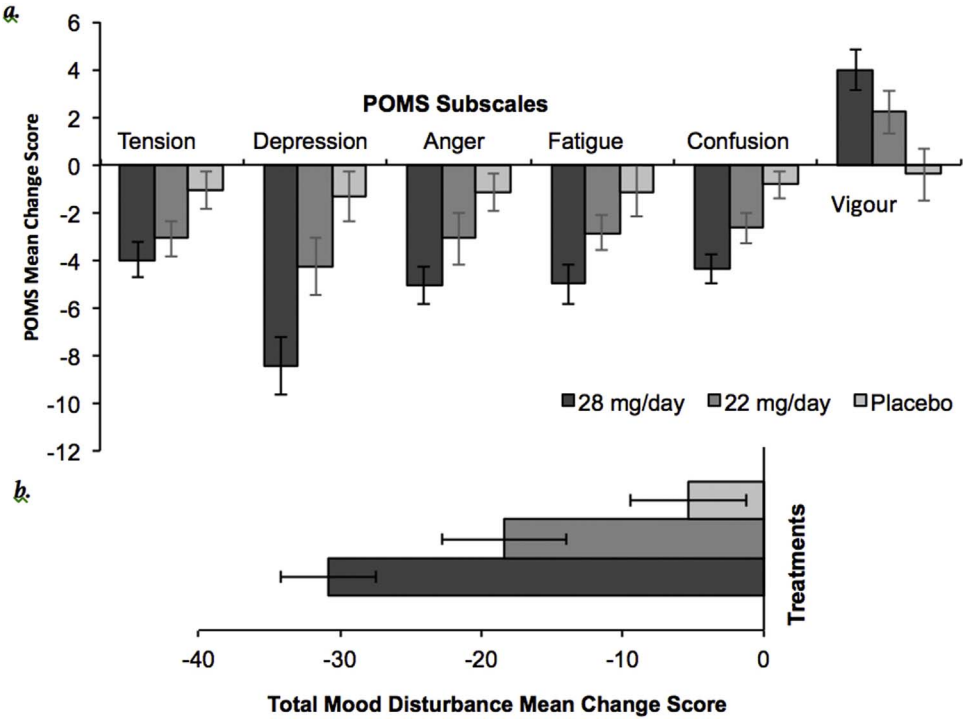

Fig. 2. A) POMS mean change scores, subscales tension, depression, anger, confusion and vigour, and (B) Total Mood Disturbance Mean Change Scores after 4 weeks of treatment with 22 or 28 mg/day affron® or placebo.

PSQ Index. There was no significant improvement in sleep quality in any of the treatment groups (Fig. 5).

3.3. Safety and tolerability and compliance

The active treatment was well tolerated. Participants returned unused containers of product at the final interview, and compliance was high and similar between all groups. Participants were monitored for adverse effects at 2 weeks and the final interview. One participant in the placebo group reported a singular event of symptoms of diarrhea.

4. Discussion

Results indicated a significant decrease in negative mood and symptoms related to stress and anxiety at a 28 mg/day dose. No significant differences were observed between the group treated with 22 mg/day of the saffron extract and the placebo group. Sleep quality showed a slight improvement at 28 mg/day dose.

The mood elevating and anxiolytic effects of affron® were consistent in both sexes, and achieved without adverse effects on any performance or safety parameters. Our results are consistent with previous studies undertaken on *Crocus sativus* L. that have shown effectiveness in alleviating the symptoms of mild to moderate depression, in some studies as effectively as fluoxetine and imipramine. [16]

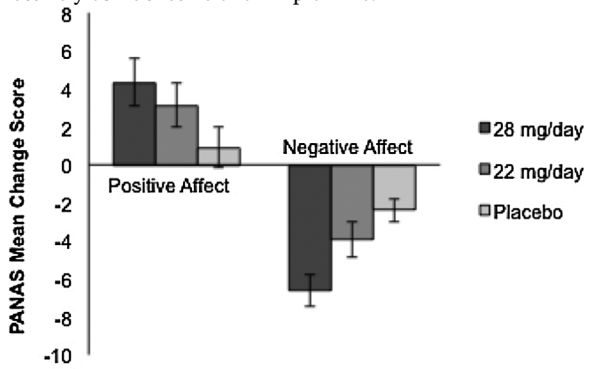

Fig. 3. PANAS Mean change scores, subscales positive affect (PA) and negative affect (NA), after 4 weeks of treatment with 22 or 28 mg/day affron® or placebo.

While we studied a population with self-reported low mood but not diagnosed with depression, our results bring new potential knowledge to the clinical literature, showing that this new standardised saffron extract exerts remarkably consistent positive effects across the POMS-TMD, PANAS and DASS scales. Furthermore, our dosing schedule demonstrated a clear dose-dependent relationship across all scales, making our study the first to identify a clinically appropriate and empirically justified dosage scheme.

To our knowledge, this is the first time that a commercial saffron extract obtained at industrial scale and objectively characterized has been tested on healthy people with a positive effect on overall mood. Given affron®'s excellent safety profile, and data indicating that low mood states may predispose to depressive illness, [13,14] it may be considered a candidate for preventative use in subjects deemed to be at risk of progressing to more severe and eventually clinical manifestations.

4.1. Limitations

The effect sizes on the outcomes in this study provided favourable results and demonstrated good internal validity; however, the study was not without its limitations. The main weaknesses of this investigation were found in the self-reporting nature of both the screening and the testing, and the possibility of confounding variables.

First, the measurement of low mood as a construct was an inexact process which relied on self-reporting of low mood at screening. The subjective nature of self-reports may have impacted on the construct

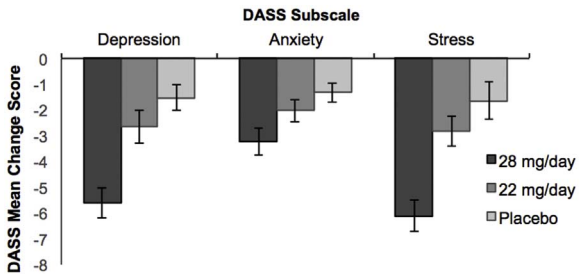

Fig. 4. DASS-21 mean change scores, subscales depression, anxiety and stress, after 4 weeks of treatment with 22 or 28 mg/day affron® or placebo.

validity of the tests by including participants who may have been excluded if a more objective screening process was employed. It is possible that participants with an undiagnosed mood disorder were included in a study that sought to exclude them.

Second, the self-reporting nature of the instruments used may have led to imprecise measures due to the subjective interpretation of items. The possibility of error could be reduced by using blood tests to measure stress hormones.

Third, while the possible confounds of BMI and gender were considered, this study did not control for other variables known to impact the outcome of mood, such as personality. [36]

Finally, to address low mood rather than clinically diagnosed disorders, this study tested a healthy population. It therefore excluded participants with a high BMI, severe PMS, insomnia, and those with a history of drug and alcohol abuse. Since these conditions are often associated with low mood, these exclusions may limit the generalisability of the study. This may be addressed by future research into saffron's efficacy for treating participants whose low mood is comorbid with more severe conditions.

5. Conclusion

Overall, the results demonstrated the effectiveness of affron®, a botanical extract from saffron (*Crocus Sativus* L.) on improving low mood, and stress in otherwise healthy participants.

Given the excellent safety profile of this food herb, the well-known issues associated with the tricyclics and SSRI's and the current absence of management tools for low mood, there is now a strong case for using saffron in the long-term and prophylactic management, where appropriate, of low mood states.

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Conflict of interest

The authors have declared there is no conflict of interest.

Authors' contributions

GK, AR, GB PC contributed to the data collection writing, data analyses and data interpretation of the clinical trial that is a part of this manuscript. AI, MG and MP conducted the laboratory analysis of the affron® samples. All the authors read and approved the final draft of the manuscript.

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