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Consumption of fresh *Centella asiatica* improves short term alertness and contentedness in healthy females

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ABSTRACT

Centella asiatica is rich in pentacyclic triterpenes that have been associated with several beneficial health effects. Several earlier studies investigated the effects of long term intake of *C. asiatica* on several cognitive functions and mood, either in the form of dried herb, powder, supplements or extract, but not as a fresh herb in a human intervention study. In this research, for the first time, the short-term effect of consuming a single smoothie, containing two concentrations of the fresh herb, on the cognition and mood of healthy female participants was investigated. Madecassic acid was the major triterpene in the fresh leaves of *C. asiatica*. Cognitive performance and mood dimensions were assessed before and one hour after consuming a single serving of smoothies. Alertness and contentedness factors significantly improved with higher concentration of *C. asiatica*. No significant improvements in cognitive functions after one hour of consumption were found.

1. Introduction

Centella asiatica (L.) Urban otherwise known as Asiatic Pennywort or Gotu Kola, belongs to the plant family *Apiaceae*, and the subfamily *Mackinlayoideae.* It grows in South-East Asian countries such as China, Sri Lanka, India, Indonesia, Malaysia, and in Africa (James and Dubery, 2009; Orhan, 2012). *C. asiatica* has been used for thousands of years as a medicinal herb in different countries and it is used as a brain food in India and as a brain tonic in Ayurvedic and Chinese medicine (Puttarak et al., 2017). *C. asiatica* is rich in pentacyclic triterpenoids mainly saponins and their aglycones, which are reportedly responsible for its beneficial effect on cognition and mood (Chandrika & Prasad Kumarab, 2015). Triterpenoid saponins are secondary plant metabolites which consist of hydrophobic triterpenes structure (aglycone) attached to a hydrophilic sugar chain (glycone) and are synthesised via the isoprenoid pathway. These pentacyclic triterpenoids are asiaticoside and madecassoside as saponins in addition to their aglycones, asiatic acids and madecassic acids (Gray et al., 2017). The four major bioactive compounds were characterized in the leaves and the aglycon form of the pentacyclic triterpenes was found to be more resistant to degradation than the glycoside form. The content of triterpene ester glycosides asiaticoside and madecassoside is at least 2% in *C. asiatica* (Puttarak et al., 2017). The presence of pentacyclic triterpenes plays a major role in its therapeutic and medicinal effects such as wound healing and memory improvements. Pentacyclic triterpenes also has been found to have antidepressive and anti-stress properties which might be due to their effect in the GABA (gamma-aminobutyric acid) system. GABA is an important inhibitory neurotransmitter and it is proven that the reduction in GABA can lead to depression and anxiety (Meeran et al., 2018).

Many researchers reported that the aqueous and ethanolic extract of *C. asiatica* regulate the synthesis of GABA in rat brains by stimulating the activity of glutamic acid decarboxylase (GAD) enzyme and these effects on GABA by *C. asiatica* might be responsible for decreasing anxiety and depression (Orhan, 2012). Moreover, the positive effect on cognition and memory of Alzheimer's patients could be due to its inhibitory effect against acetylcholinesterase (AChE). This enzyme is responsible for the reduction in the level of acetylcholine in the brain of Alzheimer's patients and the consumption of *C. asiatica* could increase the level of the acetylcholine in their brain by inhibiting (AChE). In addition, it can prevent amyloid plaque formation in the brain of people who suffer from Alzheimer's disease by modulating secretase enzymes (Sabaragamuwa et al., 2018).

In Table 1, an overview is given of studies investigating impact of C. asiatica on cognition and mood. Previous studies have investigated the therapeutic activity of *C. asiatica* in human intervention studies either as powder, capsules, supplements or water extract, but none of them tested it as fresh leaves. Most of these studies investigated the effects of long-term treatments of these preparations. Little is however known about

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the short-term effect of the single treatment on cognition and mood after a single treatment. It has been reported that twice as many females experience major depression compared to males (Kendler & Prescott, 1999) which represents a significant health imbalance. This study thus investigated the short-term effect of fresh *C. asiatica* as an ingredient in smoothies, at different concentrations, on cognition and mood of healthy female participants.

2. Materials and methods

2.1. Materials

Fresh *C. asiatica* and banana were purchased from Oriental group in Nijmegen, Netherlands while the origin of the herb is Malaysia, the source of the banana used was Colombia. All reagents used were of analytical grade. Citric acid was purchased from Iherb, HPLC grade methanol and ULC-MS grade acetonitrile were purchased from Acutu-All chemicals, Trifluoroacetic acid (TFA) was produced by J.T. Baker and sodium acetate was purchased from EMSURE®. The filters 0.45 μm were purchased from Phenomenex. Asiatic acid, madecassic acid, asiaticoside and madecassoside were purchased from Bio-connect, Folin-Ciocalteau reagent was purchased from Merck while Sodium carbonate was purchased from VWR international. All chemicals and reagents were of analytical grade.

2.2. Sample preparation

Total content of polyphenols was determined in *C. asiatica* and Banana. HPLC (high-performance liquid chromatography) was conducted to determine the exact concentration of asiatic acids, madecassic acids, madecassoside and asiaticoside in the whole aerial part of the fresh and dried samples. All samples were prepared and analysed in triplicates.

2.3. Determination of the four major compounds of pentacyclic triterpenes by HPLC

The extraction of madecassic acid, asiatic acid, asiaticoside and madecassoside was carried out using the methods described by Azwanida (2015) while the phenolic compounds were extracted from banana and fresh *C. asiatica* using a modified method described by Andrade et al. (2015). Dried and fresh herb were analysed by HPLC. The aerial part of *C. asiatica was* cut and dried at 40 °C for 24 h. Dried and fresh samples were ground. 1 g of dried powder and fresh herb were stirred with 10 ml of 100% methanol for 5 h at room temperature and filtered. The filtrate was evaporated to dryness under reduced pressure at 40 °C, using a rotary evaporator. The extract was weighed accurately by subtracting the weight of the empty flask from the weight of the flask containing the extract. The dried crude extract was dissolved in HPLC-grade methanol 100% and adjusted to 10 ml. The solution was filtered through a 0.45 μm syringe RC filter and the clear filtrate was used for HPLC analysis. Banana was also analysed by HPLC and the extraction of pentacyclic triterpenes carried out using two different methods. The first one was conducted in the same way as the herb. While the second one was done with the method described by Schaneberg et al. (2003). Banana (1 g) was extracted three times with 3 ml of methanol by sonication for 10 min then centrifuged for 5 min at 3000 rpm and supernatants combined in a 10 ml volumetric flask by pipette and diluted to the final volume with methanol and mixed. The samples were filtered through a 0.45 μm syringe RC filter before the injection. All experiments were performed in triplicate.

2.4. HPLC method

Detection and separation of pentacyclic triterpenes were performed with a reversed phase HPLC. The column used was 5 μm (C18-A), (4.6 mm × 150 mm). Mobile phase was a gradient of acetonitrile/water, and the flow rate was 1 ml/min. The optimum detection wavelength was 206 nm and the injection volume were 20 μl. The gradient condition can be found in Table 2. A stock solution of (1 mg/ml) of asiatic acid, asiaticoside, madecassic acid, and madecassoside was prepared and diluted to obtain a concentration of 4, 8, 16, 32, 63, 125, 250, 500 μg/ml.

Table 2
The amount of pentacyclic triterpenes in fresh *C. asiatica* and smoothies used in study.

Pentacyclic triterpenes	Amount of pentacyclic triterpenes (mg/g fresh herb)	Placebo	10% *C. asiatica mg/100 ml*	20% *C. asiatica mg/100 ml*
Madecassoside	0.42 ± 0.02	0	4.2	8.4
Asiaticoside	0.32 ± 0.01	0	3.2	6.4
Madecassic acid	0.56 ± 0.01	0	5.6	11.2
Asiatic acid	0.44 ± 0.01	0	4.4	8.8

Table 1
Overview of studies investigating impact of *C. asiatica* on cognition and mood.

Form of product	Dose of product	Duration of the study	Type of subjects	Observed effect	Reference
1. Capsules contain 70% hydro-ethanolic extract of dried aerial parts of CA	500 mg twice daily	2 months	33 human adults with generalized anxiety disorder, males and females	Decrease in stress-anxiety-depression related disorders- increase in attention level	Jana et al. (2010)
2. Capsules of aerial part extract of CA	250,500 or 750 mg once per day	2 months	28 human healthy elderly, males and females	Improvement in alertness and calmness-Increase in the accuracy of working memory in addition to the reduction in reaction time	Wattanathorn et al. (2008)
3. Crude stem and leaf powder dissolved in grape juice	12 g of crude stem and leaf powder	30 and 60 min	40 healthy adults, males and females	An increased self-rated energy level and a decline in acoustic startle response (ASR)	Bradwejn et al. (2000)
4. Capsules of dried 70% hydro-ethanolic whole herb extraction	1000 or 750 mg every day	6 weeks	48 human subjects with cognitive impairment	An improvement in delayed recall memory	Farhana et al. (2016)
5. Capsules of crude herb	500 mg per 10 kg body weight daily	6 months	41 healthy middle-aged adults, males and females	An increase in some cognitive functions with gender differences	Dev (2009)
6. Tablets of dried powder of whole plant	500 mg daily	3 months	30 mentally retarded children age 7–18, males and females	Increase IQ and enhanced behaviour	Appa Rao et al. (1973)
7. Juice made of fresh leaves of CA	6 ml per kg body weight	6 weeks	Healthy adult mice	Increase dendritic arborization and reduce level of β-amyloid hippocampus	Kappettu et al. (2008)
8. Ethanolic extract of CA	300 mg per kg body weight daily	60 days	Aged rats	Increase antioxidant status and decrease the level of LPO and PCO in the brain.	Subathra et al. (2005)

2.5. Human intervention study

The human intervention study was conducted to determine whether smoothies containing *C. asiatica with* two different concentrations have a positive effect on cognition and mood when compared to a placebo smoothie. Participants were informed about the study with the assurance that their participation was voluntary, they could stop at any point if they wished and their responses would be anonymously recorded. Consents were obtained from all respondents participating in the study.

2.5.1. Screening and the design of the study

Thirty female participants were recruited using an online survey distributed through social media and email. All the participants had to meet certain criteria. The participants had an average age of 25.0 ± 0.7 and all of them were females. Informed consents forms were obtained from participants (details in S1).

The intervention study was conducted in a single-blind, placebo-controlled, stratified random, cross-over design. The questionnaires for the study can be found in S2. Each participant had to come three times, once a week, to test the three different treatments. There were six different treatment orders, which were distributed randomly to the participants and each treatment order was repeated five times. There was a wash-out period of at least 3 days to make sure that all the bioactive compounds were secreted from their bodies. In each session, participants had to fill in the mood survey (Bond-Lader visual analogue scales) and to complete the BrainCheck™ cognitive test. Both were done just before and one hour after the consumption of the smoothies. The differences between scores, which were obtained from mood scales and Brain Check™ test after and before the consumption of the treatment, were calculated for each treatment and each participant. Moreover, the participants had to evaluate the sensory aspects of the smoothie, which had to be done after the consumption of the smoothies. Participants had to wait for one hour before they were asked to complete the mood survey and Brain Check™ test for the second time. The time interval of one hour was chosen as one study reported an improvement in some cognitive functions after one hour from receiving a single treatment of capsules made up of 250–750 mg *C. asiatica* extracts (Wattanathorn et al., 2008). In addition, it was suggested that the enhancement in the cognition and concentration can be noticed after 1–2 h of receiving the treatment (Udani, 2013).

The smoothies were distributed in black coffee cups with black lids and black straws in order to make sure that the participants did not notice the differences in colour and smell between them. Each participant received a cup that contained 100 ml of the smoothie per session. The smoothies were distributed with the names of the participants on the tables before the beginning of the sessions to avoid any mistakes or disturbances. Treatments and placebo smoothies with different concentration of *C. asiatica* were made to study the dose dependency of any therapeutic effect.

2.5.2. Smoothie preparation

The smoothies were made of 10 g/100 ml (low concentration) and 20 g/100 ml (high concentration) of the aerial part (leaves and stem) of fresh *C. asiatica*. Leaves and stems were chosen since the pentacyclic triterpenes that are responsible for many therapeutic effects, are mostly present in those plant parts (Puttarak et al., 2016). After the addition of 10 g banana per 100 ml to make the smoothies more palatable, and adjusting the volume with water, the mixture was blended. The placebo was prepared from just water and banana. Citric acid was added after the blending in the amount that gave the three smoothies the same level of sourness and made them as similar as possible. Both banana and citric acid made the smoothies identical in terms of texture and sweet–sour balance. Small particle size was important to ensure a similar mouthfeel and a high bioaccessibility of the pentacyclic triterpenes in the gastrointestinal tract. Banana contains sugar and tryptophan which might improve mood and cognition. Therefore, only 10 g of banana was used during the sessions which is not enough to show any positive effect since it was found that 6 g of tryptophan per day was required to reduce depression and increase concentration of human subjects, while 10 g of banana contains only about one mg of tryptophan (Richard et al., 2009). Moreover, 10 g of banana provides 1.2 g sugar while at least 25 g of sugar is needed to enhance the memory and other cognitive functions (Giles, et al., 2018). Therefore, the sugar and tryptophan content in 10 g banana were not enough to show a positive impact on cognition and mood.

2.5.3. Mood measurement tool

An online Bond-Lader Visual Analogue Scale (BL-VAS) in Qualtrics was used to measure the mood. The participants were asked to drag the slider in 100 mm line to the position that described their current mood. The mood measurement was performed by the participants before the assessment of the cognition. There were sixteen visual analogue scales anchored by antonyms, for example (strong-feeble, alert-drowsy, etc.). The scores summed up to form three different factors (alertness, calmness, and contentedness) as recommended by Bond and Lader (1974). The alertness factor consists of (alert - drowsy, attentive - dreamy, lethargic - energetic, clearheaded, well-coordinated - clumsy, mentally slow - quick witted, strong - feeble, interested - bored, incompetent - proficient).The calmness factor consists of two adjective pairs (calm-excited, tense-relaxed), the contentedness factor consists of four pairs (contented-discontented, troubled-tranquil, happy-sad, antagonistic-amicable, withdrawn-sociable) (Silvestrini et al., 2013). The scores are the distance from the positive antonyms to the participants' mark. Lower scores therefore indicate an improvement in the mood (details in S3).

2.5.4. Cognition measurement tool

Brain Check™ test was used to investigate the positive effect of the *C. asiatica* (Gotu kola) on cognition. The tool is short, easy to use and validated (Ye et al., 2020). The Brain Check™ tool includes five different tests that assess five different cognitive domains (cognitive processing, executive function, visual attention, immediate recall, and delayed recall). The tests are: Digital-symbol substitution test to measure the speed and accuracy of information processing. Stroop interference test to assess cognitive inhibition and impulse control. Trails A/B test to measure visual search speed and efficiency. Immediate recall test to measure the ability to recognize items that previously appeared. Delayed recall test to assess the ability to recognize items that previously appeared after a time delay.

2.5.5. Smoothie evaluation questionnaire

After the consumption of the smoothies, the participants were asked to fill in a smoothie evaluation questionnaire. The survey included questions about the sensory properties of the smoothies. A linear scale of five points was used. Participants were asked to rate the sourness, sweetness and bitterness of the smoothies. A high score meant that the participant found the smoothie too sour/sweet/bitter. The subjects were also asked to evaluate how much they liked the texture and the flavor of the smoothies. A mood and cognition perception questionnaire were given to the participants after the last session to find out if they expected any differences between the smoothies on mood and/or cognition (details in S6).

2.6. Statistical analysis

The results are expressed as mean values ± standard errors of the mean (SEM). The mean differences were calculated by subtracting the individual scores of the mood and cognition measurements after the consumption of the smoothies from the scores at the baseline. The data were analysed using IBM SPSS statistics 26. The normality was tested with the Shapiro-Wilk test. The outliers were checked by using the interquartile range (IQR) with a factor of 1.5. One-way ANOVA repeated measures test was used when the data was normally distributed, and it

did not contain outliers. The sphericity was measured by Mauchly's test. Post Hoc test with Tukey was conducted when the ANOVA test showed a statistical significance. When the data was not normally distributed with outliers, the non-parametric tests (Friedman test) were used, with a Wilcoxon signed-rank test when there was a significant difference between the scores. Statistical significance was set at $P < 0.05$.

3. Results and discussions

3.1. Pentacyclic triterpenes content of samples

Four pentacyclic triterpenes were found in the fresh samples: madecassoside, asiaticoside, madecassic acid and Asiatic acid but none was detected in banana (Table 2). The pentacyclic triterpenes composition of the three smoothies used in the intervention study were based on the composition of *C. asiatica* and banana is also given in Table 2.

3.2. Mood measurements of participants

The scores of the mean differences of the scores obtained from BL-VAS scales before and after the consumption were normally distributed with no outliers and the assumption of sphericity was met for the three mood factors. The smoothies with 20% of *C. asiatica* gave a significant improvement of the alertness factor compared to placebo (Fig. 1). The 10% smoothie scored in between the placebo and 20% smoothie, suggesting a dose–response effect. The smoothie with 20% of C. asiatica was able to improve the contentedness factor compared to placebo and the 10% smoothie. No significant difference in the calmness factor between the three smoothies was observed.

The improvement in the alertness factor was in agreement with other (long term) studies on *C. asiatica*. Wattanathorn et al., 2008 also used Bond-Lader visual analogue scales to assess the mood of elderly volunteers before and after one and two months of the treatment with *C. asiatica* using a standardized aerial part extract of *C. asiatica* in form of capsules with different doses (250, 500 and 750 mg). The extract contained 1.09 mg/g and 48.89 mg/g of asiaticoside and asiatic acid respectively. The capsules of 750 mg improved the alertness factor significantly after one and two months of the treatment in comparison to placebo, while the calmness factor was improved significantly after the consumption of the capsules of all doses.

After investigating the effect of *C. asiatica* on the three factors of mood, it was interesting to study how the underlying sixteen adjective word pairs of the mood questionnaire were affected by the smoothies. Since the scores were not all normally distributed or/and some word pairs contained too many outliers, the Friedman test was conducted to compare the mean differences after and before the consumption of the three smoothies (S3).

Four of the sixteen adjective pairs of the mood (muzzy-clear headed, well-coordinated- clumsy, contend-discontented and antagonistic-amicable) showed a significant reduction in the mean differences after drinking the smoothie with 20% of *C. asiatica* compared to 10% and placebo (Figs. 2a and 2b). For instance, the participants became more clear-headed after the consumption of the 20% smoothie (-6.86 ± 3.11) in comparison with placebo (22.26 ± 8.60), which was statistically significant ($p = 0.007$). Furthermore, there was a significant difference between the scores of the 10% smoothie and placebo for (clear-headed - clumsy). This means that a low concentration C. *asiatica* smoothie (10 g/100 ml) was already able to make the participants feel more clear-headed in comparison with the placebo drink.

In terms of scores of contended - discontented (Fig. 3), there was a significant ($p = 0.009$) decline in the mean difference following the consumption of the smoothie containing high quantity of *C. asiatica* (-15.46 ± 6.69), while the score was higher after the consumption of the placebo (25.06 ± 9.38). Furthermore, the participants became more contended after drinking high concentration smoothie in comparison with low concentration one. There is also a significant difference between the scores of amicable and antagonistic after consuming the low concentration and high concentration smoothies, the mean difference of the former one (5.33 ± 3.00) is higher than the latter (-8.80 ± 3.81), which means the participants felt more amicable and less antagonistic after drinking the 20% *C. asiatica* smoothie. The mean difference of the 20% smoothie was lower than the placebo smoothie and they showed a significant difference between them ($P = 0.011$) for (amicable-antagonistic). The mean difference of the scores obtained from the rest of adjective pairs of the mood did not show any significant differences between the smoothies, even though there were a decrease in some of these scores after the consumption of *C. asiatica* smoothie. For example, the mean difference of the scores of adjective pair (Energetic-lethargic) was (-5.46 ± 3.93) and (-1.86 ± 4.49) after drinking high and low concentration smoothies, respectively, compared to placebo (19.56 ± 7.90), but they were not statistically significant. One possible reason that there was a negative effect on the mood after consuming placebo might be due to participants getting tired during waiting before the second test. This was not the case with smoothies that contain *C. asiatica*. The reason could be that *C. asiatica* has an adaptogenic activity with potential action in the regulation of hypothalamopituitary-adrenocortical axis (HPA axis) which helps to inhibit stress in the consumer (Jana et al., 2010). Therefore, it might be suggested that *C. asiatica* increased the ability of the participants to manage the tensions and stress during the one hour waiting time. It has also been reported that the adaptogens of *C. asiatica* during the stress situations play an important role in the reduction of cortisol and nitric oxide (Panossian, 2017). This occurs due to its content of pentacyclic triterpene that has an impact on the HPA axis. However, the exact mechanisms by which *C. asiatica* enhances the mood need further investigation.

In the study by Udani (2013), the profile of mood status (POMS) test

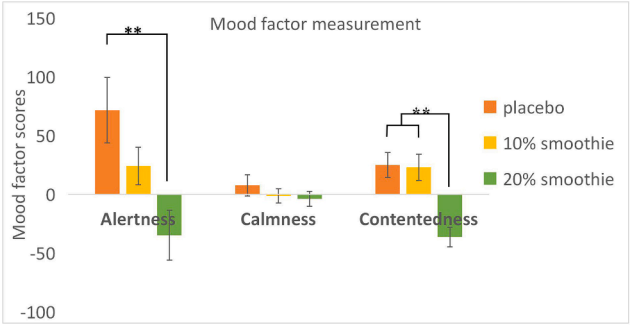

Fig. 1. Mean differences of the mood factor scores after and before the consumption of the smoothies (±SEM). Lower scores indicate an improvement in the mood factor. **$p < 0.01$.

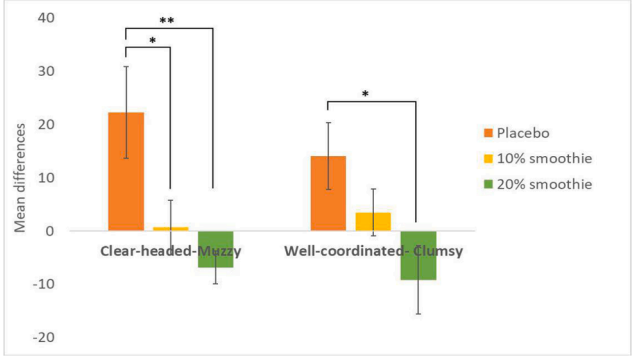

Fig. 2a. The mean differences of the scores ± (standard error of the mean) of two adjective pairs of the mood that showed a significant difference in alertness factor between the smoothies. Low or negative scores indicate an improvement in the mood factor $P < 0.01$.

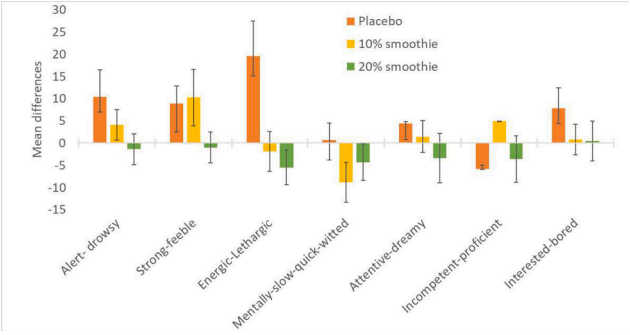

Fig. 2b. The mean differences of the scores ± (standard error of the mean) of some adjective pairs of the mood. Low or negative scores indicate an improvement in the mood factor $P < 0.01$.

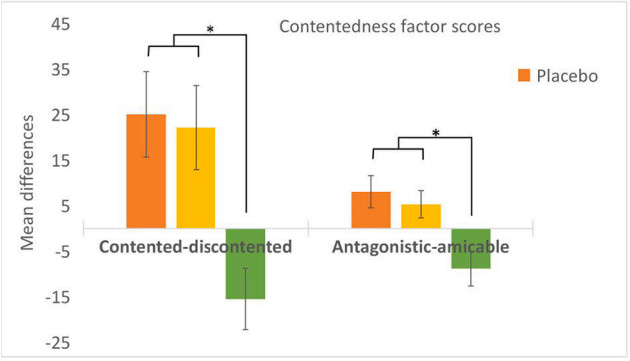

Fig. 3. The mean differences of the scores ± (standard error of the mean) of two adjective pairs of the mood that showed a significant difference in Contentedness factor between the smoothies. Low or negative scores indicate an improvement in the corresponding mood factor $P < 0.01$.

was performed to measure the mood after taking 100 mg *C. asiatica* extract in the form of capsule with a mixture of additional different natural ingredients such as 50 mg turmeric extract. A significant decrease in tension, depression, and anger was observed after 5 h of the consumption of the single dose, but no improvements were recorded in the mood after one hour. It is difficult to predict which ingredients were responsible for the positive effect on the mood after 5 h of consuming a single treatment since the capsule contained a combination of different medicinal extracts.

3.3. Cognition measurements

Friedman test was conducted on the results obtained from the cognition test (BrainCheck) as there were several outliers. There was no significant difference between the treatments and placebo on any of the cognitive domains (Table 3). The mean difference of immediate recall showed an improvement after the consumption of the smoothie with 20% of *C. asiatica* in comparison with the smoothie of 10% of herb and placebo, but they were not statistically significant ($P > 0.05$) as can be seen in Table 3. All three smoothies had a negative effect on delayed recall. This could be because the delayed recall test was the last one in Brain Check™ tests and the participants could have been tired.

Table 3
Mean differences of the scores ± (standard error of the mean) of cognitive domain obtained from Brain Check™ test.

Cognitive domain	Placebo	Low concentration	High concentration
Cognitive processing	3.30 ± 1.72	0.06 ± 1.44	2.93 ± 1.30
Executive function	−1.97 ± 1.59	0.53 ± 1.45	1.03 ± 1.56
Visual attention	0.97 ± 1.53	3.97 ± 1.56	1.23 ± 1.72
Immediate recall	−1.77 ± 2.03	−1.83 ± 2.23	2.17 ± 2.04
Delayed recall	−3.67 ± 2.31	−5.13 ± 3.68	−2.73 ± 1.41

Previous research done by Wattanathorn et al. (2008) reported an improvement in the reaction time of spatial memory and the accuracy of numeric working memory in the healthy elderly after one hour of the consumption of 750 mg of capsules containing extract of *C. asiatica*. On the other hand, even though the smoothie with 20% of the herb had higher amount of asiaticoside (6.4 mg) than the capsule of C. asiatica extract which contained 0.8 mg asiaticoside, it did not show an improvement in the cognition after one hour. This could be because asiaticoside need more than one hour to be transformed into asiatic acid by the enzymes since it was found that there was a delay in the availability of asiatic acid in the blood after the consumption of asiaticoside, while asiatic acid was available in the plasma shortly after consuming a pure asiatic acid (Rush et al., 1993). Another reason for not having an improved cognition function might be that a single dose was not enough to show an enhancement on cognition since it was reported that a chronic treatment of *C. asiatica* resulted in a higher concentration of asiatic acid in the plasma and therefore more pronounced effect compared to a single treatment (Grimaldi & De Ponti, 1990). The polyphenols content in the smoothie with 20% of *C. asiatica* was higher than the capsule of 750 mg *C. asiatica* (29 mg and 22 mg, respectively), but it did not improve the cognition after a single treatment. Short term effect of a capsule containing 100 mg of C. asiatica extract with a mixture of other natural extracts such as 50 mg of turmeric extract was investigated by (Udani, 2013). A significant increase in an executive functioning was however reported after one hour of the consumption of the capsules despite the capsule containing lower amount of *C. asiatica* as compared to the smoothie with 20% of the herb. Executive functioning however improved after one hour of the consumption. This could be because the other medicinal extracts of the capsules also contribute to the development in the executive functioning together with *C. asiatica*. After 5 h, there was an increase in reaction time, working memory, cognitive flexibility and sustained attention compared to placebo but these improvements were not attributed to only *C. asiatica* since it was combined with other ingredients and it was difficult to predict which one was responsible for the enhancement. It was reported by Wattanathorn et al. (2008) that the acute positive effect of *C. asiatica* on cognition after a single treatment could happen by regulating dopamine and norepinephrine with adjusting of acetylcholine and serotonin in prefrontal cortex and hippocampus, respectively, whereas the improvement in the mood is a result of different mechanism such as the reduction in nitric oxide or the modulation of GABA synthesis in the brain (Provino, 2010). Therefore, it might be suggested that the quantity of bioactive compounds in the smoothie was effective to trigger the mechanism that improves the mood, but it was not enough to have a positive impact on the memory and other cognitive functions.

3.4. Smoothie evaluation

There was a significant difference in the bitterness between the placebo and the *C. asiatica* smoothies, which was expected because the herb is bitter, and the placebo contained only banana and water. As seen in Fig. 4, the bitterness scores of the smoothie with 20% of *C. asiatica* is higher than the scores of the smoothie with 10% of the herb and placebo. On the other hand, the subjects were not able to distinguish between the smoothies in terms of the sourness because a similar amount of the citric acid was added to make the three smoothies as similar as possible. Furthermore, participants could not find any differences in the sweetness between the drinks because banana was added in equal amount to give the three smoothies the same texture and to make them more palatable by adding sweet taste. The participants liked the texture of the placebo more than the texture of the other smoothies since the particle size of the herb was bigger than the one obtained after blending banana.

To ensure bias was removed in the study, participants were

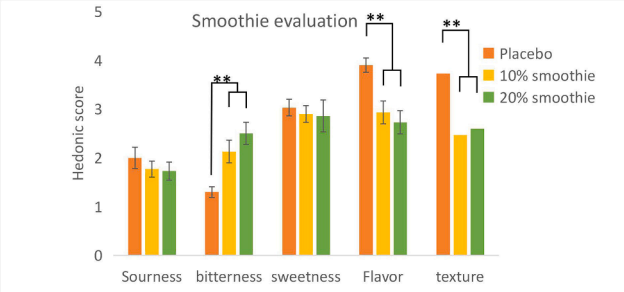

Fig. 4. Smoothie evaluation results ± (standard error of the mean) obtained from the survey that was done after the consumption of the smoothies.

separately asked if they noticed any differences between the smoothies that they consumed. Nine participants thought that the smoothie with 10% of the herb had a positive impact on their mood and ten believed that the drink with 20% of *C. asiatica* improved their mood and concentration. On the other hand, placebo was able to improve the mood of five participants while six participants did not notice any improvements in their mood or cognition after drinking the smoothies. There was however a negative effect of the placebo on the alertness and contentedness factors even though the participants liked its texture and flavor more than other smoothies.

4. Conclusion

Consuming a smoothie containing *C. asiatica was* found to have a positive impact on alertness and contentedness compared to placebo, while calmness was not affected. This study is the first that reports the enhancement in alertness and contentedness already one hour after the consumption of fresh *C. asiatica*. Four adjective pairs of the mood, (clear headed-muzzy and well-coordinated - clumsy) related to the alertness factor and (contented-discontented and amicable-antagonistic) from the contentedness factor, demonstrated a significant improvement after drinking the smoothie with 20% of *C. asiatica* as compared to placebo. The smoothie with 10% of the herb was able to improve the adjective pair (clear headed-muzzy) of alertness factor after one hour in comparison to the placebo. The presence of pentacyclic triterpenes mainly asiatic acid and asiaticoside is expected to play a major role in these improvements, but the mechanism requires further investigation. In contrast to previous long term studies and one short term treatment of *C. asiatica* in combination with other ingredients, none of the smoothies improved the cognitive functions (cognitive processing, executive function, visual attention, immediate recall and, delayed recall). The possible reason that the cognition was not improved could be that a single treatment of *C. asiatica and* the interval time of one hour after the consumption were not enough to show a positive impact on cognition. Novel food products containing *C. asiatica* as an ingredient could be utilized as a mood food.

Ethics statement

Informed consent for experimentation with human subjects

In compliance with ethical standards, consents were obtained from all respondents participating in the study (S1) Participants were informed about the study with the assurance that their participation was voluntary, they could stop at any point if they wished and their responses would be anonymously recorded.

Authors' contributions

All authors provided feedback on the manuscript and approved the submitted version.

CRediT authorship contribution statement

Oluranti Mopelola Lawal: Conceptualization, Investigation, Writing - original draft. **Fatima Wakel:** Investigation. **Matthijs Dekker:** Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104337.

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