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Advanced Nutraceutical Approach for Mood/Calmness, Sleep, and Skin Health in Moderately Stressed Adults: A Randomized, Double-blind, Placebo-controlled Study

Shelly N Hester[1], Steven M Wood[1], Rebecca Major[1], Tanner Gibb[1], Melanie Riggs[1], Marilyn Contreras Pinegar[1], Helen Knaggs[1], Ganesh Diwakar[1], Barrie Drewitt[2], Edwin D Lephart[3*]

[1]Pharmanex Research, NSE Products, Inc. Provo, Utah, USA

[2]Princeton Consumer Research Corp. Chelmford, UK CM2 6UA

[3]Department of Cell Biology, Physiology and The Neuroscience Center, College of Life Sciences Brigham Young University , Provo, Utah, USA*

Abstract

Introduction: The recent reported link between the brain, gut and skin axis where the neuro-dermal unit serves as a "neuro-endocrine-immune" organ demonstrates the complex network involved in human health and disease. Several investigations have examined individual active ingredients in nutraceuticals, but little information is available for combination supplementation with multiple active ingredients. Thus, the effects of lemon balm extract, magnolia bark extract and L-theanine on stress, sleep quality and skin health were examined in moderately stressed adults in this randomized, double-blind, placebo-controlled investigation.

Methods: Sixty moderately stressed adults 25 to 55 years of age were randomized [CS group (n = 30) or placebo group (n = 30)] and consumed two capsules daily to deliver 600 mg of lemon balm extract, 40 mg of honokiol (*via* Magnolia bark extract), and 200 mg of L-theanine for 30 days. Subjects were assessed using the Hamilton Anxiety Rating Scale (HAM-A), Hamilton Depression Rating Scale (HDRS), a Restorative Sleep Questionnaire (RSQ-W), and a self-perception of skin attributes questionnaire.

Results: The CS group displayed significant improvement over the placebo group for anxiety (HAM-A) and depression (HDRS) plus enhanced sleep quality and several skin attributes. No adverse events were reported, and the CS was well tolerated.

Conclusions: This clinical study demonstrates that short-term consumption of a combination supplement composed of lemon balm, magnolia bark and L-theanine extracts underscores the therapeutic potential to improve mood/calmness, sleep quality, and skin health. Further research is warranted to confirm these findings in longer-term studies.

Keywords: Stress, Sleep, Skin, Lemon balm, Honokiol, L-theanine

Graphic Abstract

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Introduction

Nutraceuticals are formulated to achieve recommended dietary requirements, offer preventive benefits, and/or improve overall health. Several investigations have examined individual active ingredients in nutraceuticals, but little information is available for combination supplementation with multiple active ingredients. Some of the most important health parameters involve stress, sleep quality, and skin well-being, especially with aging [1].

Stress is a biological response to any external or internal stimuli which results in alterations in the homeostatic state, affecting physical, physiological and cognitive wellbeing [2]. While stressors such as physical exercise or calorie restriction help to build adaptation to stress stimulus, chronic or long-term stressors can lead to deterioration of physical, physiological and cognitive function as well as disturbing the microbiota of the gut-immune-brain axis [3]. Studies have shown that increased stress, reduced sleep, and disturbances in circadian rhythms impact not only specific melatonin receptors in the skin but cause the acceleration of skin aging [4].

Stress management strategies include getting adequate sleep, engaging in regular exercise, cultivating positive social relationships, and eating a well-balanced diet [1]. In this regard, active ingredients in dietary supplements have been shown to produce meaningful and significant modulation of stressors that influence multiple health factors such as anxiety, depression, sleep quality, and skin health [1].

Lemon Balm Extract (LBE) (*Melissa officinalis*), a member of the mint family, has been used for centuries to reduce stress, anxiety, improve sleep, and relieve discomfort from indigestion [5]. LBE is composed of flavonoids (quercetin, rhamnocitrin, and luteolin), polyphenols (rosmarinic acid, caffeic acid and chlorogenic acid), triterpenes (ursolic acid and oleanolic acid) and essential oils as active constituents [6]. A systematic review and meta-analysis demonstrated the significant positive effects of LBE on depression and anxiety [5]. The mechanism for LBE's reduction of anxiety, depression and increase in sleep quality is by enhancing the action of GABA, the brain's major inhibitory neurotransmitter [5]. Thus, LBE generates a calming effect by inhibiting the enzymatic activity of GABA transaminase that breaks down GABA to increase GABA levels in brain, which can relieve anxiety, epilepsy, and related neurological disorders [5]. LBE has also been shown to have positive skin effects upon oral intake as it contains phytochemical compounds that protect the skin from oxidative stress via solar Ultraviolet Light (UV), improve skin elasticity, and prevent glycation (i.e. the non-enzymatic glycosylation of proteins that cause tissue and collagen damage) [6].

Magnolia Bark Extract (MBE) (*Magnolia officinalis*) is a flowering herb that comes from the houpo magnolia tree [7]. The main phytochemicals from MBE are the polyphenolic neolignans magnolol and honokiol [7]. MBE has been used in traditional Chinese medicine to treat anxiety, depression, stress, nervousness, sleep related problems, inflammation/oxidative stress and dermatological disorders [7]. Mechanistically, these benefits are likely due to MBE's potent antioxidant and anti-inflammatory activity [7], as well as its effects on enhancing GABA neurotransmitter action, blocking NfkB and COX-2 for neuroprotection, increasing brain serotonin levels [7], and activating Nrf2, which is the master switch to turn on cellular defense, antioxidants and detoxifying enzymes [7]. For example, Tabbott et al. showed that MBE decreased cortisol and improved psychological mood in moderately stressed individuals [8].

L-theanine (L-T) is an amino acid present in black, green and white teas (Camellia sinensis) with health benefits through its antioxidant activity, anti-inflammatory effects, neuroprotective effects and positive effects on dermal wellness, sleep, and mood [9]. For the mechanisms of mood and neuroprotection, L-theanine acts as an antagonist at the binding sites on the presynaptic membrane of neurons, which inhibits glutamine transporters and blocks the reuptake of glutamine and glutamate, thereby inhibiting the conversion of glutamine to glutamate, which is the primary excitatory neurotransmitter in the brain [9]. Chronic stress and alternations in mood can affect the glutamatergic system in the brain where excess levels of glutamate can cause damage to neurons [9]. Topical delivery of L-theanine can ameliorate acute skin inflammation, and oral administration of L-theanine (in a randomized controlled study) along with ashwagandha and saffron showed improvement in fatigue, mood, sleep quality and skin health biomarkers after 8 weeks of treatment [10].

Finally, the recent link between the brain, gut and skin axis where the skin serves as a "neuro-endocrine-immune" organ demonstrates the complex network involved in human health and disease [4,11]. Especially since advances in microbial research have linked chemical messengers (hormones/neurotransmitters) to the brain-gut-skin microbiome to influence brain, gut and dermal wellbeing [11,12].

While there are numerous anti-stress extracts available, based upon the traditional/extended use, safety and efficacy profiles these herbal remedies were selected for this investigation [13-15] furthermore, to our knowledge, no previous human study has quantified the combined effects of LBE, MBE, and L-T on stress, sleep quality, and skin health. Therefore, the primary objective of this clinical study was to assess the efficacy of a unique dietary Combination Supplement (CS), composed of 600 mg of LBE, 40 mg of honokiol from MBE, and 200 mg of L-T on stress, while the secondary objectives included CS's influence on sleep and skin parameters as well as confirming safety/tolerance of oral administration in moderately stressed adults.

Materials and Methods

Ingredient Sources and Chemical Analysis

The chemical analysis of the active ingredients in the combination supplement have been reported and are covered in

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brief below.

For example, Lemon Balm (*Melissa officinalis* L.) from Nexira (Rouen, France) was extracted with 450 m/l of aqueous ethanol by medium pressure-liquid-solid extraction and subsequently analyzed by HPLC yielding hydroxycinnamic acid derivative, flavonoids with caffeic acid, m-coumaric acid, eriodictyol-7-O-glucoside, naringin, hesperidin, rosmarinic acid, naringenin and hesperetin being identified based on their chromatographic behavior and spectral analysis [16].

The main chemicals from the magnolia bark extract (*M. officinalis*) from Guangxi, China are the neolignans magnolol and honokiol that are found in the highest concentrations of the roots of the trees by extraction with ethanol and water [17]. Magnolol and honokiol have been quantified by HPLC with Ultraviolet (UV) detection at 290 nm [17]. In this case, 100 mg of magnolia bark extract was standardized to contain 40 mg of honokiol, as determined by HPLC using internal controls.

For L-Theanine, this amino acid present in natural plant products was isolated by a fermentation process initially with corn protein from Ethical Natural (San Anselmo, CA, USA), then purified through cellulose and micron filters and the extract obtained was analyzed by HPLC on a C-18 column with absorbance at 195 nm at 20 minutes [18].

Supplement Composition

Combination supplement (CS): The combination supplement delivered 600 mg of Lemon Balm Extract (LBE), 100 mg of magnolia bark extract (containing 40 mg of honokiol) (MBE), and 200 mg L-theanine (L-T) daily in 2 capsules, taken with water.

Placebo (PL): The placebo delivered excipients only (stearic acid and natural caramel color to match the appearance and weight (mg) of the CS capsule) that were taken as 2 capsules daily with water.

Clinical Study Design and Ethical Considerations

A priori power analysis was performed to estimate the required sample size. The study was conducted in accordance with the ethical principles of the 1975 declaration of helsinki and its subsequent amendments. This was a single-center, double-blind, randomized study conducted at Princeton Consumer Research (PCR) Corp. in Essex, United Kingdom between July 2023 and August 2023. The CONSORT protocol was used for trial findings and reporting (Figure 1).

The study was sponsored by Pharmanex Research, NSE Products Inc., Provo, Utah, USA and the College of Life Sciences at Brigham Young University, Provo, Utah, USA, but the sponsors or company representatives had no contact/interactions with the study participants. The duration of the study was thirty days, with 3 in-clinic visits.

This clinical study with Princeton Consumer Research (Study Number No. RIGCL122) was approved by the East Anglia Ethics Committee, UK on 19th June 2023. The subjects signed informed consent forms, met all the inclusion criteria (and none of the exclusion criteria) and were subsequently enrolled for this study, which was conducted between July and August 2023. Additionally, this randomized, double-blind, placebo-controlled study was registered in the clinicaltrials.gov database (NCT06672965).

Figure 1: Schematic CONSORT flow diagram demonstrating participant enrollment in the study..
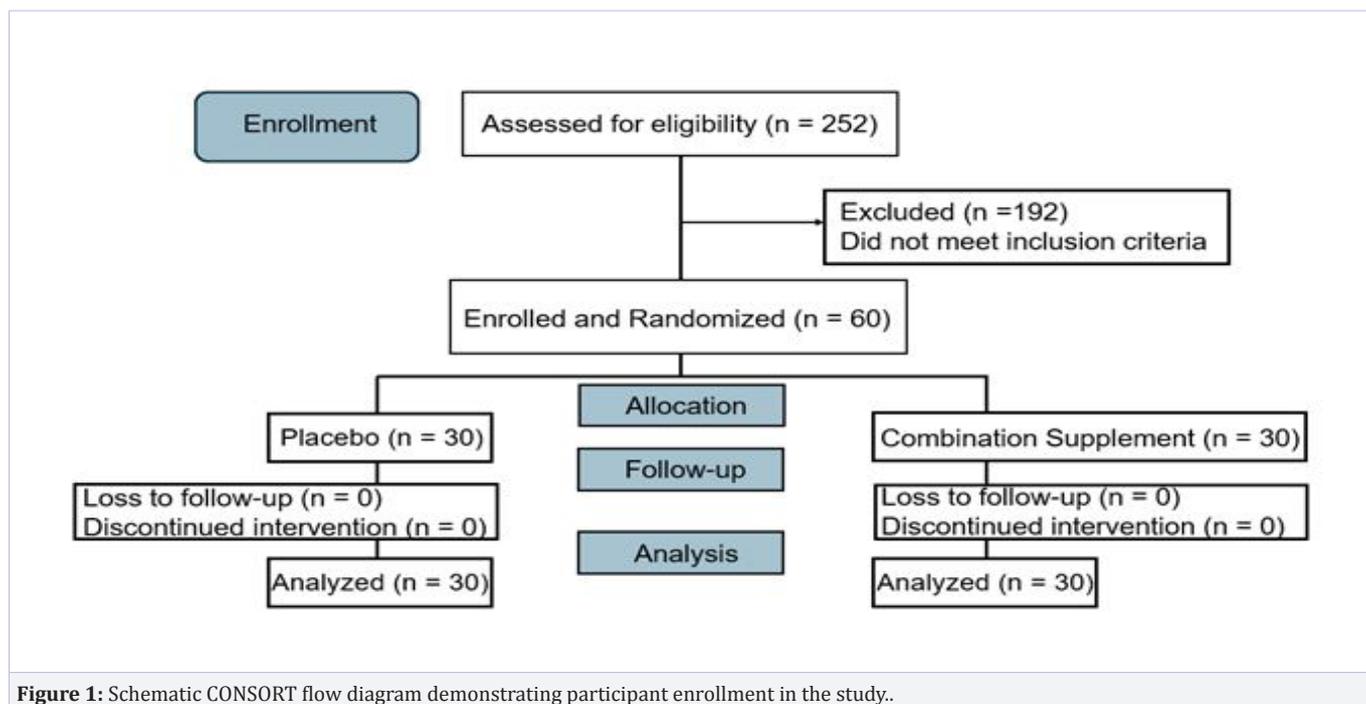
Participants

A total of sixty adults (including male and female subjects) were enrolled, randomized and completed this study. The study inclusion criteria included: moderately stressed subjects with a minimum score > 14 according to the Perceived Stress Scale (PSS) as reported by Simon in 2021 and having no reported history of psychological or mental disorders [19]. Additionally, the inclusion criteria covered the following: The subject was a healthy male or female 25-55 years of age at the time of informed consent. The subject must have a Body Mass Index (BMI) of > 18.5 and < 30.0 kg/m^2 and be willing to have their height and weight measured. Free of anti-depressants/anti-anxiety/mood enhancing/ recreational drugs (e.g., marijuana, Tetrahydrocannabinol (THC), Cannabidiol (CBD) use for at least 6 months prior to screening. Free of any 'over the counter' or prescribed medications except for female participants taking contraceptive pills; agree to abstain from smoking, vaping, or using recreational drugs (e.g., marijuana, THC, CBD) during the study and finally, willing and able to give informed consent to participate in the study.

The exclusion criteria included: having a resting systolic blood pressure above 140 mmHg or a diastolic pressure above 90 mmHg at baseline and taken dietary supplements or pharmaceuticals within the last 3 months to help relieve sleep disturbances or physiological stress, having taken anti-depressants/anti-anxiety/mood enhancing/recreational drugs (e.g., marijuana) or having smoked or vaped recreational drugs within 6 months prior to screening. Also, having any known chronic skin conditions, pregnant or planning to become pregnant or nursing or individuals that had used certain medications, which in the opinion of the investigators may interfere with the study. Subjects were between 25 to 55 years of age with a Body Mass Index (BMI) of >18.5 and <30.0 kg/m^2. Subjects enrolled in the study were randomized to the Combination Supplement (CS) group ($n = 30$) or the Placebo Group (PL) ($n = 30$). Baseline characteristics and demographics of the subjects are shown in Figure 2.

Subjects were given 60 capsules of either the combination supplement or placebo (30-day supply) and instructed to take two capsules each day orally with water. All subjects enrolled completed the study. To avoid bias, subjects and researchers were blinded to the CS and placebo treatments, and the containers were coded by a third party. Thus, this study was carried out in a double-blind manner. Finally, there were no specific dietary guidelines given to the test subjects nor did the subjects record their daily food intake so the potential impact of diet on the obtained results was not considered.
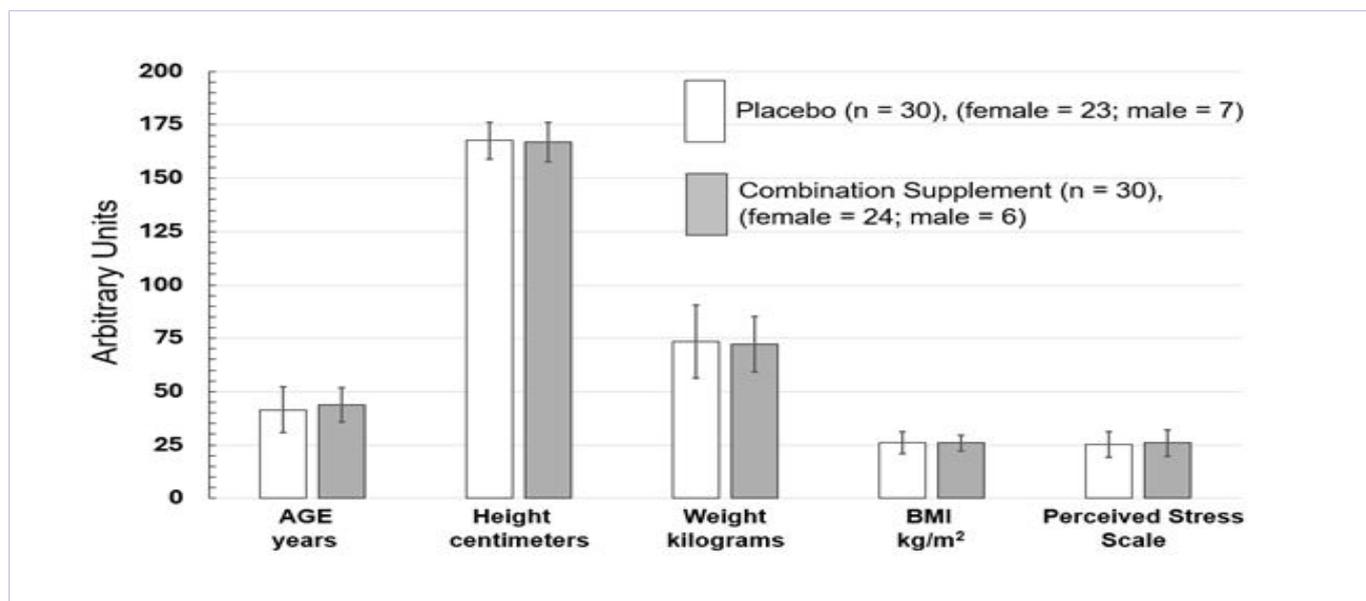
Figure 2: Baseline characteristics of the subjects by treatment group.
(Kg = Kilograms, m = Meters and Perceived Stress Scale was above 14 for both treatment groups as a measure of stress perception at the moderate level *via* the 10-item inventory [19].

Measured Parameters and Schedule of Assessment

According to the outline of schedule assessment(s) of the quantified parameters (Figure 3), subjects were interviewed by a registered social worker and scored on the Hamilton Anxiety Rating Scale (HAM-A) [20] and the Hamilton Depression Rating Scale (HDRS) [21]. They also completed the Restorative Sleep Questionnaire (RSQ-W) [22], and a self-perception of skin attributes assessment form [23]. The skin attributes were self-assessed by each subject via a sponsor-developed questionnaire using a scale of 1 to 10 (1 = Severe/Poor Conditions, 10 = Excellent/Perfect Condition). Also, subjects collected salivary samples in provided Salivettes (Sarstedt tubes, Leicester, UK) in the morning post-awakening (within 1 hour of awakening) and evening (within 1 hour before going to sleep) at baseline and day 29. The saliva samples were placed into the subject's home

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refrigerator overnight, and then delivered to the clinical test facility the next day, where all the saliva samples were stored at 40 °F (4.4 °C) until all samples were assayed with Elecsys®Corisol II (Electrochemiluminescence immunoassay) "ECLIA" method (Roche Diagnostics Mat. No. 06687733160, Burgess Hill, UK).

Quantified Parameters	Baseline	Day 1	Day 7	Day 29	Day 30
Salivary Cortisol collected	X			X	
Hamilton Anxiety Rating Scale (HAM-A)	X				X
Hamilton Depression Rating Scale (HDRS)	X				X
Restorative Sleep Questionnaire (RSQ-W)	X	X	X		X
Skin Attributes Assessment Questionnaire (Self – Perception)	X	X	X		X

Figure 3: Schedule of assessment of the quantified parameters. The X indicates the baseline and days after treatment the various parameters were quantified.

Statistical Analysis

Data was analyzed using the Wilcoxon signed ranked test, which is a non-parametric statistical method to determine if there is a significant difference between paired samples (e.g., before/after measurements from the same subject) and the data does not follow a normal distribution [24]. The differences are ranked and then used to determine the test statistic (W). If the test statistic (W) is less than or equal to the critical value, then a significant difference between the pair has a significant effect. This test is commonly used for drug intervention studies. For example, in the present study, where comparisons were made between baseline vs. 30 days values for each of the measured parameters by treatment group and CS vs PL group; ($p < 0.05$) was considered statistically significant. Finally, for each data set tested in figures 4-7 the statistical test is stated along with the p value or the precision of the statistical test result(s).

Results

HAM-A (anxiety) and HDRS (depression) Scale Assessment

The HAM-A scores (measure of anxiety) were quantified at day 30 and compared to baseline scores by treatment group. On day 30, HAM-A scores improved in 100% of the subjects in the CS group, compared to only 21% of subjects in the PL group. When the HAM-A mean scores were analyzed, daily CS supplementation significantly reduced HAM-A scores compared to the PL group, indicating reduced anxiety, as shown in Figure 4.

The HDRS scores (measure of depression) were quantified at day 30 and compared to baseline scores by treatment group. The HDRS scores improved in 100% of the subjects in the CS group compared to only 21% of subjects in the PL group. When the HDRS-mean scores were analyzed, daily CS supplementation significantly reduced HDRS scores compared to the PL group, indicating reduced depression, as shown in Figure 5.

Overall, the results indicated that daily consumption of the CS significantly improved anxiety and depression scores compared to the PL group values after 30 days. No significant improvement in HAM-A (anxiety) and HDRS (depression) scores were observed in the PL group on day 30.

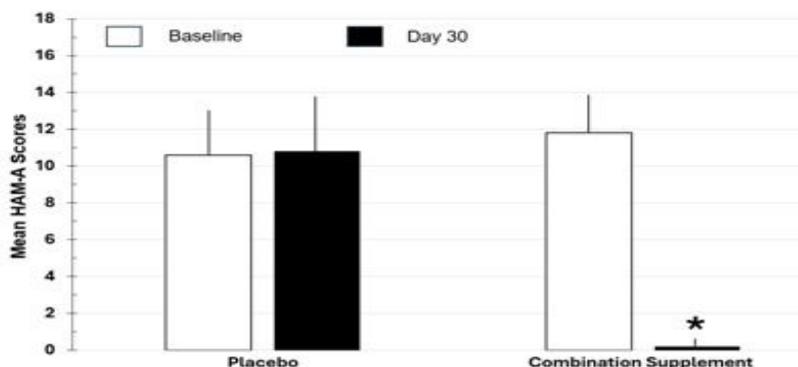### Restorative Sleep Questionnaire (RSQ-W) Assessment

Assessment of CS versus PL was done on sleep quality using the Restorative Sleep Questionnaire (RSQ-W) [22], which measures to what extent subjects felt tired, sleepy, rested, refreshed/restored, ready to start the day, energetic, mentally alert, grouchy, or in a good mood.

The CS group displayed significantly greater sleep quality as measured by RSQ-W scores after one day on the CS (an increase of 16.4% over baseline levels) versus the PL group (an increase of 9.7% over baseline levels) ($p < 0.02$). Moreover, the CS group showed significantly greater sleep improvement over baseline at day 7 (86.1%, $p < 0.01$) and day 30 (86.8%, $p < 0.01$), which was also significantly greater compared to the PL values at day 7 (23.1% over baseline) or day 30 (30.5% over baseline) ($p < 0.01$ and $p < 0.02$ respectively, CS *vs* PL). Finally, the percentage of subjects within the CS group that improved on day 7 was 86.7%, and 90.0% improved on day 30. In comparison, the percentage of subjects within the PL group that improved on day 7 was 53.9% and 73.1% on day 30.

Skin Attributes Self- Perception Assessment

Using the self-perception of skin attributes assessment form, subjects performed a self-assessment using a scale of 1 to 10 (1 = Severe/Poor Condition, 10 = Excellent/Perfect Condition). The skin attributes for this assessment included: brightness, smoothness, radiance/glow, softness, skin tone evenness, appearance of dark circles, hydration, and overall appearance [23].

Daily consumption of the CS resulted in significant

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Figure 4: Hamilton anxiety rating scale scores influenced by daily consumption of a Combination Supplement (CS) *versus* Placebo (PL) supplement. Values represent the Mean HAM-A scores ± standard deviation. * = Significantly decreased anxiety levels in the CS *vs*. the PL group ($p < 0.01$); *p*-values were calculated with the Wilcoxon signed ranked test comparing data between the CS and PL groups after 30 days. On average, subjects in the CS group had a score of 11.8 at baseline that significantly decreased to 0.2 on day 30 (a 98.3 % decrease in anxiety levels, $p < 0.01$). In contrast, the PL group's baseline score of 10.6 increased slightly to 10.8 (a non-significant increase of 2.3 %, $p = 0.16$).
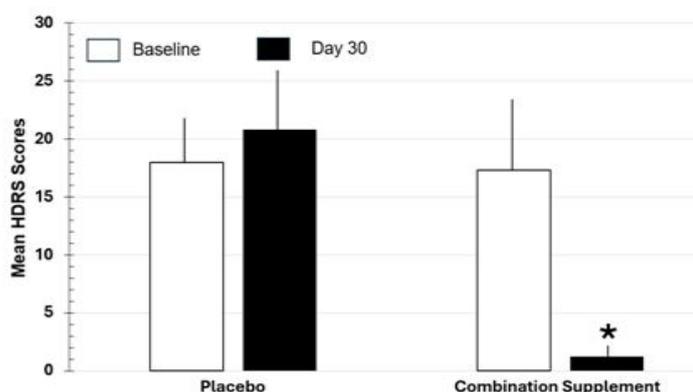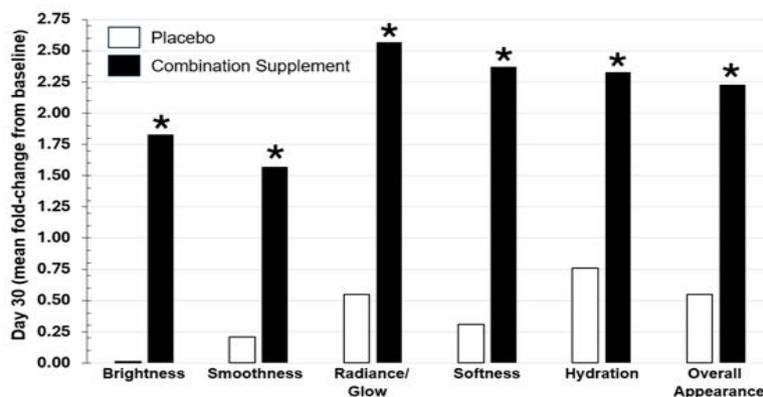

Figure 5: Hamilton Depression Rating Scale (HDRS) scores influenced by daily consumption of a Combination Supplement (CS) versus Placebo (PL) Supplement. Values represent the Mean HDRS scores ± standard deviation. * = Significantly decreased depression levels in the CS *vs*. the PL group ($p < 0.01$); *p*-values were calculated with the Wilcoxon signed ranked test comparing data between the CS and PL groups after 30 days. On average, subjects in the CS group had a score of 17.3 at baseline that significantly decreased to 1.2 on day 30 (a 93.2 % decrease in anxiety levels, $p < 0.01$). In contrast, the PL group's baseline score of 18.0 increased slightly to 20.8.

improvements in skin attributes, including brightness, smoothness, radiance/glow, softness, hydration and overall appearance compared to baseline values and the scores from PL group at day 30, as shown in Figure 6.

Salivary Cortisol Assessment

Cortisol is a key biomarker for the induction (and relief) of stress [8]. In this study, salivary cortisol levels were determined by immunoassay of samples collected in the morning and the evening at baseline and on day 29 by treatment group. The salivary cortisol data was higher in the morning versus the evening in both groups, but there were no significant differences in the values in the groups at baseline or at day 29 (Figure 7).

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Figure 6: Self-reported skin attributes. The data represents the mean fold-change at day 30 from baseline values. * = Significantly greater improvement in skin parameters in the CS group compared to baseline levels or PL values at day 30 (*p* < 0.05). The data were calculated using the Wilcoxon signed ranked test comparing baseline data *vs* day 30 values and the results obtained between the treatment groups (*p* < 0.05).

Treatment	Baseline AM	Day 29 AM	Baseline PM	Day 29 PM
Combination Supplement	3.4 ± 1.9	3.9 ± 2.7	1.2 ± 0.9	1.8 ± 1.9
Placebo Control	3.9 ± 2.5	3.5 ± 1.9	1.4 ± 1.2	1.2 ± 0.9

Figure 7: The Influence of the combination supplement and placebo control values on salivary cortisol levels (ng/ml) during treatment. Cortisol values are presented as the mean ± standard deviation. There were no significant differences in cortisol levels by treatment group.

Discussion

Lemon Balm Extract (LBE) [6] and Magnolia Bark Extract (MBE) have been used for centuries to treat a variety of health aliments, while L-theanine (L-T) (discovered in 1949 by Yajiro Skakato in Kyoto, Japan) has also been reported to ameliorate high blood pressure and promote relaxation [7-10]. Previous investigators have shown the positive benefits of LBE, MBE and L-T, where in most studies these active ingredients were examined individually on such disorders such as mood, anxiety, stress, insomnia, and for skin health [5,9,10,25]. Notably, all three ingredients (LBE, MBE and L-T) in this report have been shown to cross the blood-brain-barrier supporting their positive neurobehavioral actions and health benefits [5].

For example, LBE has been shown to improve mood, decrease stress and anxiety by increasing brain GABA neurotransmitter action [5,26], boost cognitive function [27], decrease insomnia [26], and increase sleep quality [21] as well as help relieve indigestion and protect against solar UV light to improve skin elasticity and prevent glycation that leads to tissue and collagen damage [6].

Furthermore, MBE has been shown to improve mood, acting as an anti-depressant, anti-anxiety, and anti-stress agent by boosting GABA, activating cannabinoid receptors, serotonin, and dopamine while inhibiting adrenaline [7,8] and decrease cortisol levels in stressed individuals [8]. It also has anti-inflammatory actions due to its antioxidant properties to activate Nrf2 to decrease oxidative stress [7], and it promotes increased sleep quality [28,29].

Finally, L-T has been shown to enhance mood [10], decrease stress and anxiety [9,10], prevent skin inflammation [10], increase sleep duration and NREM sleep [29] and decrease blood pressure under physical and psychological stress [30].

It is important to note the Combination Supplement's (CS) effects and obtained results of the present study may offer health benefits other than those described above. To illustrate this point, the three ingredients (LBE, MBE and L-T) may work together to produce the significant improvements in depression, anxiety, sleep quality, and enhanced skin health, and/or work synergistically because the short-term (30-day treatment) was effective compared to longer intervals of administration(s) (8 to 12 weeks) in previously reported clinical studies of the individual ingredients [31].

It is well established that stress and sleep influence skin health [1] as well as poor sleep increases risk of disease and illness [1]. Even a single night of disrupted sleep can influence anxiety/stress and dermal inflammation plus alter the clock genes. These clock genes are active during the day to provide skin protection, while at night the clock genes function to increase skin maintenance and repair, which have been reviewed elsewhere [1]. Thus, in brief, twenty health parameters aided by LBE, MBE and L-T are summarized in figure 8. Thus, combining LBE, MBE and L-T improved parameters of anxiety, depression and skin attributes potentially by increasing antioxidant levels through Nrf2, increased serotonin and dopamine levels.

Health Conditions or Factors	Lemon Balm Extract (LBE)	Magnolia Bark Extract (MBE)	L-Theanine (L-T)
1. Enhance Mood	●	●	●
2. Decrease Anxiety	●	●	●
3. Decrease Stress	●	●	●
4. Increase Calmness	●	●	●
5. Enhance GABA	●	●	●
6. Decrease Glutamate			●
7. Activate Cannabinoids Receptors			●
8. Increase Serotonin		●	
9. Modulate Dopamine		●	
10. Boost Cognitive Function	●	●	●
11. Inhibit Adrenaline		●	
12. Anti-inflammatory Actions	●	●	●
13. Antioxidant Actions	●	●	●
14. Activate Nrf2		●	
15. Decrease Oxidative Stress	●	●	●
16. Increase Sleep Quality/Duration	●	●	●
17. Improve General Skin Health	●	●	●
18. Protect Skin from Ultra Violet Light	●		
19. Decrease Blood Pressure			●
20. Relieve Indigestion	●		

Figure 8: Health conditions & factors aided by Lemon Balm Extract (LBE), Magnolia Bark Extract (MBE) and L-Theanine (L-T) as active ingredients in nutraceutical supplements. GABA = Gamma Amino Butyric Acid the major inhibitory neurotransmitter in the brain and spinal cord; Glutamate is the primary excitatory neurotransmitter in the brain; Cannabinoid receptors in the brain are known to relieve pain, reduce inflammation and elevate mood; Serotonin and dopamine are two brain neurotransmitters that are important in improving mood and depression; Adrenaline is a hormone strongly associated with stress; Anti-inflammatory actions include inhibiting Nuclear Factor Kappa B (NFkB) a master-switch for inflammation or a key mediator of inflammatory responses; Nuclear factor erythroid 2-related factor 2 (Nrf2) helps cells respond to oxidative stress and toxic stress by increasing the expression of metabolizing enzymes that helps detoxify and also stimulates the production of antioxidants; Oxidative Stress is a condition that occurs when the cells have too many free radicals and not enough antioxidants to neutralize them, and if unchecked, can lead to tissue and cellular damage; Ultra Violet (UV) light can damage skin cells, cause sunburn and damage skin DNA molecules.

Limited clinical studies have investigated a combination supplement containing the active ingredients that were combined in the CS in the present study. Recall, in the present randomized, double-blind, placebo-controlled study of the short-term (30 day) oral treatment by the nutraceutical active ingredients, LBE, MBE, and L-T, significantly improved anxiety and depression parameters (improved mood and generated calm), sleep quality, and six different skin biomarkers of dermal wellbeing in moderately stressed adults. Where available, previous clinical data are reviewed and compared to the present clinical findings.

Kennedy et al. in separate clinical studies (in randomized, placebo-controlled, double-blind investigations) examined lemon balm's anti-anxiety influence and stress-reducing effect and found improved modulation of mood and cognitive function [32,33] when orally administered at either 300 mg or 600 mg (or in various dosing regimens). These clinical studies demonstrated consistent results in calmness, alertness, mood and cognitive function and have been reviewed elsewhere [34]. Other investigators showed that lemon balm supplements decreased stress and anxiety and improved sleep quality, when lemon balm was administered at 600 mg for 15 days or 400 mg for 3 weeks, respectively [29,32].

Ghazizadeh et al. reported a systematic review and meta-analysis of the effects of lemon balm on depression and anxiety, which suggested this may be an effective treatment to improve anxiety and depressive symptoms [5]. Other clinical studies found that a combination of valerian and lemon balm was effective in the treatment of restlessness and insomnia in children [5,33,34]. Also, valerian and lemon balm were an effective intervention for sleep disorders during menopause [34]. In general, the present findings confirm and extend previous investigations on the positive neurobehavioral, sleep, and skin benefits of Lemon Bark Extract (LBE).

Fewer clinical studies have been conducted on Magnolia Bark Extract (MBE) and L-Theanine (L-T). The therapeutic applications of compounds in the Magnolia family have been reviewed [35,36]. One pilot, double-blind, placebo-controlled clinical trial in healthy women showed that administration of 250 mg capsules of MBE and phellodendron extract (taken 3 times daily) for 6 weeks significantly decreased anxiety levels (as measured by the Speilberger anxiety questionnaire), while salivary cortisol levels were not significantly altered between the treatment group compared to placebo values [35]. However, the report by Talbott et al. showed that ingestion of 500 mg daily of a supplement containing magnolia bark extract and phellodendron extract significantly reduced stress, mood, fatigue, and salivary cortisol levels after 4 weeks of treatment [8]. Of note, both previous clinical MBE supplement studies administered 5 times the dose of total MBE compared to the present study of 100 mg MBE, where mood and depression were significantly improved but salivary cortisol (AM) levels were not significantly changed in the CS vs. the placebo-control group. In general, the positive influence of MBE on neurobehavioral parameters was confirmed in the present study, however, the lower dose of MBE may account for no changes in salivary cortisol level.

Previous randomized, double-blind, placebo-controlled clinical trials have examined L-Theanine (L-T). One study in 2011 by Ritsner et al. showed that 400 mg per day of L-T for 8 weeks significantly reduced anxiety and general psychopathology parameters in patients with schizophrenia [37]. Also in 2011,

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Lyon et al. reported that 400 mg of L-T for six weeks significantly improved several aspects of sleep quality in boys with Attention Deficit Hyperactivity Disorder (ADHD) [38]. Finally, in 2019 Hidese et al. examined the effects of oral ingestion of 200 mg of L-T per day for 4 weeks on stress symptoms, sleep parameters, and cognitive function in healthy adults, where all endpoints were significantly improved including verbal fluency and the ability to stay focused [39]. Collectively, these previous clinical reports employed L-T levels that were 2-4 times higher compared to the present clinical study, however, the current results are comparable for significant improvement in mood/calm, stress-related symptoms and sleep quality, which may be due to the CS pleotropic actions to enhance the health parameters.

Of course, the present study is not without limitations. The make up of the study sample had more women compared to men, although, women are nearly twice as likely as men to be diagnosed with mood/depression and affective disorders [40,41]. While it was the aim of the study to investigate the beneficial effects of the active ingredients in the combination supplement compared to placebo-controls, the inclusion and exclusion criteria themselves were not based on formal psychological diagnostic criteria. However, this study did determine pre-study stress levels in the subjects via the Perceived Stress Scale (PSS) [19]. The effectiveness of the combination supplement presumably is due to the supportive antioxidant and anti-inflammatory actions of the active ingredients, but the mechanisms of how this may occur, especially the potential synergistic effects, were not elucidated in the present study. Finally, no dietary factors were controlled nor were the participants instructed to follow any specific dietary guidelines, thus food intake did not bias the outcome of this study.

In summary, the combination of lemon balm extract, magnolia bark extract, and L-theanine on lowering stress parameters is consistent with the functional activity reported in the literature on these individual ingredients on improving parameters of anxiety, depression, and sleep.We extend the literature by demonstrating *in vivo* self-reported skin benefits, as well as proposing that this daily supplement can be used to support feelings of calmness in individuals with moderate daily stress.

Conclusion

Lifestyle stress in urban and rural societies related to economic and social causes has been shown to significantly impact wellbeing and overall health. There is an unmet need to manage mild to moderate stress which, if not managed properly in the initial stages, could lead to more severe and long-term stress. The results from this 4-week study support and extend previous reports on the benefits of lemon balm extract, magnolia bark extract, and L-theanine and demonstrate that relatively low dosing of these active ingredients in a combination supplement can significantly improve mood/calm, sleep quality, and skin parameters by working together presumably *via* synergistic mechanisms. The nutraceutical combination supplement was well-tolerated, and there were no reported adverse events/effects. Based upon this study, further clinical nutraceutical research

is warranted to reveal the beneficial effects of combination supplementation, where regimen treatments and/or aesthetic, functional, and system prevention plans may become standard options to improve health and wellness.

Disclosure Statement

There are no prior publications or submissions with any overlapping information, including studies and patients.The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.SNH, SMW, RM, TG, MR, M C-P, HK and GD were employed by NSE Products, Inc. BD is employed by PCR Corp. in Chelmford, UK. However, SMW, M C-P and GD are no longer with NSE Products. Finally, the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors Contributions and Credit of Authorship

SNH, SMW, RM, TG, MR M C-P, HK, GD, BD and EDL made substantial contributions to the concept, design, data analysis, text writing, editing and draft preparation. All authors were involved in reviewing the manuscript for publication, approved the final version and are accountable for the accuracy of the data presented.

Shelly N. Hester: design, writing, methodology, data analysis. Steven M. Wood: design, writing, methodology, data analysis. Rebecca Major: provide project supervision and editing and writing. Tanner Gibb: Validation, draft design, methodology. Melannie Riggs: study supervision, data and statistical analysis. Marilyn Contreras-Pinegar: concept development, administrative support, analysis. Helen Knaggs: design, review & editing, administrative supervision, funding acquisition. Barrie Drewitt: clinical study director, project manager/director of clinical research. Ganesh Diwakar: design, methodology and data analysis, writing & editing. Edwin D. Lephart: concept development, administration for funding acquisition, writing, editing, organization and overall research project supervision.

Ethical Approval and Subject Consent

1. Research involving humans: Ethical approval for the involvement of human subjects in this study was granted by the East Anglia Ethics Committee, UK on 19th June 2023 and this

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study was conducted by Princeton Consumer Research (Study Number No. ROGCL122)

2.Written informed consent was obtained from each subject for conducting this randomized, double-blind, placebo-controlled clinical study for publication of this report

Data Availability Statement

The raw data collected for this study can be requested subject to approval by contacting the corresponding author

Reference

1. Helen Knaggs, Edwin D Lephart. Enhancing skin anti-aging through healthy lifestyle factors. Cosmetics. 2023; 10(5); 142. doi.org/10.3390/cosmetics10050142

2. Tsigos C, Kyrou I, Kassi E, Chrousos GP. Stress: Endocrine physiology and pathophysiology, In: Feingold KR, Anawalt B, Blackman MR, editors. Endotext, South Dartmouth, USA, 2020

3. Stephen C Bondy. The hormesis concept: strengths and shortcomings. Biomolecules. 2023; 13(10): 1512. doi: 10.3390/biom13101512.

4. Ying Chen, John Lyga. Brain-skin connection: Stress, inflammation and skin aging. Inflamm Allergy Drug Targets. 2014; 13(3): 177-190. doi: 10.2174/1871528113666140522104422

5. Javid Ghazizadeh, Saeed Sadigh Eteghad, Wolfgang Marx, Ali Fakhari, Sanaz Hamedeyazdan, Mohammadali Torbati, et al. The effect of lemon balm (Melissa officinalis L.) on depression and anxiety in clinical trials: A systematic review and meta-analysis. Phytother Res. 2021; 35(12): 6690-6705. doi: 10.1002/ptr.7252

6. Muhammad Arshad Ullah, Ali Hassan. Medicinal benefits of lemon balm (Melissa officinalis) for human health. World Journal of Chemical and Pharmaceutical Sciences. 2022; 1(1): 28-33. doi.org/10.53346/wjcps.2022.1.1.0025

7. Anna Woodbury, Shan Ping Yu, Ling Wei, Paul Garcia. Neuro-modulating effects of honokiol: a review. Front Neurol. 2013; 4: 130. doi: 10.3389/fneur.2013.00130

8. Shawn M Talbott, Julie A Talbott, Mike Pugh. Effect of Magnolia officinalis and Phellodendron amurense (Relora®) on cortisol and psychological mood state in moderately stressed subjects. J Int Soc Sports Nutr. 2013; 10(1): 37. doi:10.1186/1550-2783-10-37

9. Liwen Wang, Margaret Brennan, Shiming Li, Hui Zhao, Klaus W Lange, Charles Brennan. How does the tea L-theanine buffer stress and anxiety. Food Science and Human Wellness. 2022; 11(3): 467-475. doi.org/10.1016/j.fshw.2021.12.004

10. Jessica Maloh, Mincy Chakkalakal, Fatima Sulaiman, Waqas Burney, Cindy J Chambers, Raja K Sivamani. Combining topical and oral botanicals for skin redness, pigmentation, sleep and mood: A randomized controlled study. J Clin Med. 2022; 11(22): 6690. doi: 10.3390/jcm11226690

11. Radomir M Slominski, Chander Raman, Anton M Jetten, Andrzej T Slominski. Neuro-immuno-endocrinology of the skin: how environment regulates body homeostasis. Nature Rev Endocrinol. 2025; doi.10.1038/S41574-025-01107-x.

12. Edwin D Lephart, Frederick Naftolin. Estrogen action and gut microbiome metabolism in dermal health. Dermatol Ther (Heidelb). 2022; 12(7): 1535-1550. doi: 10.1007/s13555-022-00759-1

13. Imogen Maria Mathews, Jessica Eastwood, Daniel Joseph Lamport, Romain Le Cozannet, Pascale Fanca Berthon, Claire Michelle Williams. Clinical efficacy and tolerability of lemon balm (Melissa officinalis L.) in psychological well-being: A Review. Nutrients. 2024; 16(20): 3534. doi: 10.3390/nu16203545

14. Andrea Sarrica, Natalja Kirika, Margherita Romeo, Mario Salmona, Luisa Diomede. Safety and toxicology of magnolol and honokiol. Planta Med. 2018; 84(16): 1151-1164. doi: 10.1055/a-0642-1966

15. Marc Moulin, David C Crowley, Lora Xiong, Najla Guthrie, Erin D Lewis. Safety and efficacy of alphawave® L-theanine supplementation for 28 days in healthy adults with moderate stress: A randomized, double-blind, placebo-controlled trial. Neurol Ther. 2024; 13(4): 1135-1153. doi: 10.1007/s40120-024-00624-7

16. Keyvan Dastmalchi, H J Damien Dorman, Paivi P Oinonen, Yusrida Darwis, Into Laakso, Raimo Hiltunen. Chemical composition and in vitro antioxidative active of a lemon balm (Melisssa officinalis L.) extract. LWT-Food Sci Tech. 2008; 41(3): 391-400. doi.org/10.1016/j.lwt.2007.03.007

17. Iris Usach, Alessandro Alaimo, Juan Fernandez, Alessandro Ambrosini, Sara Mocini, Lacramioara Ochiuz, et al. Magnolol and honokiol: Two natural compounds with similar chemical structure but different physiochemical and stability properties. Pharmaceutics. 2021; 13(2): 224. doi: 10.3390/pharmaceutics13020224

18. Matteo Perini, Silvia Pianezze, Luca Ziller, Federica Camin. Characterization of L-theanine in tea extracts and synthetic products using stable isotope ration analysis. J Food Drug Anal. 2021; 29(2): 311-318. doi: 10.38212/2224-6614.3349

19. Patricia D Simon. The 10-item perceived stress scale as a valid measure of stress perception. Asia Pac Psychiatry. 2021; 13(2): 12420. doi.10.111/1pply.12420. doi: 10.1111/appy.12420

20. M Hamilton. The assessment of anxiety states by rating. Br J Med Psychol. 1959; 32(1): 50-55. doi: 10.1111/j.2044-8341.1959.tb00467

21. M Hamilton. A rating scale of depression. J Neurol Neurosurg Psychiatry. 1960; 23(1): 56-62. doi: 10.1136/jnnp.23.1.56

22. Christopher L Drake, Ron D Hays, Robert Morlock, Fong Wang, Richard Shikiar, Lori Frank, Ralph Downey, et al. Development and evaluation

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of a measure to assess restorative sleep. J Clin Sleep Med. 2014; 10(7): 733-741. doi: 10.5664/jcsm.3860

23. Shelly Hester, Steven Wood, Russell Gray, Dale Kern, Zoe Draelos. Collagen supplement improves skin attributes in healthy adults. Curr Dev Nutr. 2021; 5(Suppl 2): 321. doi: 10.1039/cdn/nzab037 031

24. Wilcoxon F. Individual comparisons by ranking methods. Biometrics. 1945; 1: 80-83

25. Jackson L Williams, Julian M Everett, Nathan M D'Cunha, Domenico Sergi, Ekavi N Georgousopoulou, Richard J Keegan, et al. The effects of green tea amino acid L-theanine consumption on the ability to manage stress and anxiety levels: A systematic review. Plant Foods Hum Nutr. 2020; 75(1): 2-23. doi: 10.1007/s11130-019-00771-5

26. Andrew Scholey, Amy Gibbs, Chris Neale, Naomi Perry, Anastasia Ossoukhova, Vanessa Bilog, et al. Anti-stress effects of lemon balm-containing foods. Nutrients. 2014; 6(11): 4805-4821. doi: 10.3390/nu6114805

27. Catherine Ulbricht, Thomas Brendler, Joerg Gruenwald, Benjamin Kligler, David Keifer, Tracee Rae Abrams, et al. Lemon balm (Melissa officinalis L.): an evidence-based systematic review by the natural standard research collaboration. J Herb Pharmacother. 2005; 5(4): 71-114.

28. Breus MJ. Is magnolia bark extract the missing link for your sleep and health? Psychol Today. 2018

29. Suhyeon Kim, Kyungae Jo, Ki Bae Hong, Sung Hee Han, Hyung Joo Suh. GABA and L-theanine mixture decrease sleep latency and improves NREM sleep. Pharm Biol. 2019; 57(1): 65-73. doi: 10.1080/13880209.2018.1557698

30. Ai Yoto, Mao Motoki, Sato Murao, Hidehiko Yokogoshi. Effects of L-theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. J Physiol Anthropol. 2012; 31(1): 28. doi: 10.1186/1880-6805-31-28

31. Joanna Sutkowska, Natalia Hupert, Katarzyna Gawron, Jakub W Strawa, Michał Tomczyk, Antonella Forlino, et al. The stimulating effect of rosmarinic acid and extracts from rosemary and lemon balm on collagen type I biosynthesis in osteogenesis imperfecta type I skin fibroblasts. Pharmaceutics. 2021; 13(7): 938. doi: 10.3390/pharmaceutics13070938

32. D O Kennedy, G Wake, S Savelev, N T J Tildesley, E K Perry, K A Wesnes, A B Scholey. Modulation of mood and cognitive performance following acute administration of a single dose of melissa officinalis (lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. Neuropsychopharmacology. 2003; 28(10): 1871-1881. doi: 10.1038/sj.npp.1300230

33. David O Kennedy, Wendy Little, Andrew B Scholey. Attenuation of laboratory-induced stress in humans after acute administration of Melissa officinalis (Lemon Balm). Psychosomatic Med. 2004; 66(4): 607-613. doi: 10.1097/01.psy.0000132877.72833.71

34. Imogen Maria Mathews, Jessica Eastwood, Daniel Joseph Lamport, Romain Le Cozannet, Pascale Fanca-Berthon, Claire Michelle Williams. Clinical efficacy and tolerability of lemon balm (Melissa officinalis L.) in psychological well-being: A review. Nutrients. 2024; 16(20): 3545. doi: 10.3390/nu16203545

35. Fatima Khatoon, Sabeeha Ali, Vijay Kumar, Abdelbaset Mohamed Elasbali, Hassan H Alhassan, Salem Hussain Alharethi, et al. Pharmacological features, health benefits and clinical implications of honokiol. J Biomol Struct Dyn. 2023; 41: 7511-7533. doi: 10.1080/07391102.2022.2120541

36. Ming Yue Li, Hong Yan Liu, Ding Tao Wu, Ahmad Kenaan, Fang Geng, Hua Bin Li, et al. L-Theanine: A unique functional amino acid tea (Camellia sinensis L.) with multiple health benefits and food applications. Front Nutr. 2022; 4: 9: 853846. Doi:10.3389/fnutr.2022.853846

37. Michael S Ritsner, Chanoch Miodownik, Yael Ratner, Tatyana Shleifer, Maria Mar, Leonid Pintov, et al. L-theanine relieves positive, activation, and anxiety symptoms in patients with schizophrenia and schizoaffective disorder: an 8-week, randomized, double-blind, placebo-controlled, 2-center study. J Clinical Psychiatry, 2011; 72(1): 34-42. doi: 10.4088/JCP.09m05324gre

38. Michael R Lyon, Mahendra P Kapoor, Lekh R Juneja. The effects of L-theanine (Suntheanine®) on objective sleep quality in boys with attention deficit hyperactivity disorder (ADHD): a randomized, double-blind, placebo-controlled clinical trial. Alternative Med Rev. 2011; 16(4): 348-354.

39. Shinsuke Hidese, Shintaro Ogawa, Miho Ota, Ikki Ishida, Zenta Yasukawa, Makoto Ozeki, et al. Effects of L-theanine administration of stress-related symptoms and cognitive functions in healthy adults: A randomized controlled trial. Nutrients. 2019; 11(10): 2362. doi: 10.3390/nu11102362

40. Paul R Albert. Why is depression more prevalent in women?. J Psychiatry Neurosci. 2015; 40(4): 219-221. doi: 10.1503/jpn.150205

41. Vivian Li, Kelly Frasier, Emily Woolhiser, Kathleen Daly, Sara Christoforides, Courtnee Harpine, Karina Stech, Stefany Acosta, Edwin D Lephart. Exploring the intersection of body dysmorphic disorder (BDD) and dermatological conditions: A Narrative review. Dermatol Ther (Heidelb). 2024; 14: 2693-2708. doi: 10.1007/s13555-024-01256-3