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Research Article

Banana Peel Extract (Happy Banana®) Promotes Circadian Rhythm Regulation and Sleep Health: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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ABSTRACT

Banana peel, a common agricultural by-product, was found to be rich in polyphenols, flavonoids, and amine compounds with potential neuroregulatory effects. This study aimed to investigate the effects of banana peel extract (Happy Banana®) on sleep-related metabolic and physiological mechanisms. Initially, the active compound Hydroxyanigorufone was identified and shown to significantly upregulate the expression of key enzymes in the tryptophan metabolic pathway *in vitro*. A randomized, double-blind, placebo-controlled clinical trial was subsequently conducted over four weeks, enrolling 50 subjects with self-reported sleep disturbances. The banana peel extract showed potential benefits in improving sleep quality, although it did not demonstrate a significant additional advantage over placebo. However, the results demonstrated that subjects in the banana peel group exhibited a significant reduction in the Low-Frequency/High-Frequency (LF/HF) ratio of heart rate variability by weeks 2 and 4, indicating improved autonomic balance. Serum serotonin and salivary melatonin levels increased significantly by 13.9% and 43.3%, respectively, and the night-to-day melatonin concentration differential increased by 209%, suggesting enhanced circadian rhythm alignment. Subjective assessments revealed a 13.9% reduction in daytime drowsiness and a 19.0% decrease in nocturnal urination frequency. No abnormalities were observed in routine hematological or biochemical parameters, supporting the extract's favorable safety and tolerability profile. These findings supported its potential use as a natural sleep aid and highlighted the value of upcycling agricultural waste into functional health products.

Keywords: Autonomic nervous system; Banana peel; Melatonin; Serotonin; Sleep

INTRODUCTION

Insomnia is a highly prevalent sleep disorder that affects approximately one-third of the adult population worldwide. Chronic sleep disturbances, including insufficient sleep duration and poor sleep quality, are associated with adverse health outcomes and decreased quality of life [1]. These disturbances are also linked to impaired attention, cognitive decline, reduced occupational performance, and an increased risk of accidents, which collectively contribute to significant societal and healthcare burdens [1]. Accumulating evidence suggests that prolonged sleep deprivation is closely associated with the development of metabolic conditions such as type 2 diabetes, obesity, and hypertension, as well as cardiovascular diseases and mood disorders including depression [2]. Furthermore, sleep disorders have been implicated in a higher incidence of traffic accidents, workplace injuries, and overall mortality [3]. Epidemiological surveys indicate that approximately 10–15% of

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adults experience moderate to severe sleep-related problems, with prevalence increasing to over 25% among the elderly [4]. In primary care settings, insomnia is reported in as many as 69% of patients, particularly among those with chronic medical conditions [5].

Common pharmacological treatments for insomnia include benzodiazepines, non-benzodiazepines benzodiazepine receptor agonists, antihistamines and sedative antidepressants. While these medications may offer short-term relief, long-term use is often associated with adverse effects such as memory impairment, daytime drowsiness, increased risk of falls, tolerance, and dependence [6]. Consequently, the development of alternative therapies that are both safe and effective for long-term use has become a growing area of interest. These include botanical extracts, functional foods, and specific nutrients. From a physiological perspective, tryptophan is an essential amino acid that must be obtained through the diet [7]. Once it crosses the blood-brain barrier, tryptophan is converted by Tryptophan Hydroxylase (TPH) into serotonin (5-hydroxytryptamine), and subsequently into melatonin by Hydroxyindole-O-Methyl Transferase (HIOMT) in the pineal gland [8]. Serotonin plays a central role in regulating mood and anxiety, and modulates the sleep–wake centers in the hypothalamus and brainstem [9]. Melatonin, in turn, is a critical hormone involved in the regulation of circadian rhythms and the initiation of sleep [10]. The synthesis of serotonin and melatonin is influenced by various factors including light exposure, physiological stress, and dietary intake. Inefficient nocturnal conversion of serotonin to melatonin can disrupt circadian rhythms and contribute to difficulty initiating or maintaining sleep [11]. Thus, supplementation with tryptophan-rich compounds or botanical agents that enhance melatonin biosynthesis has emerged as a promising strategy for improving sleep quality.

Banana peel (*Musa spp.* peel) is a nutrient-rich plant-based by-product that contains a variety of bioactive compounds, including polyphenols, flavonoids, γ-aminobutyric acid (GABA), and tryptophan [12]. These constituents are known to exhibit potential neuroregulatory and sleep-promoting effects. Tryptophan is an essential amino acid that serves as a precursor for the biosynthesis of serotonin and melatonin, both of which are involved in regulating mood, circadian rhythms, and the sleep–wake cycle [13]. Its metabolic pathway involves several key enzymes, including Tryptophan Hydroxylase (TPH), aromatic L-amino acid Decarboxylase (DDC), and Arylalkylamine N-acetyltransferase (AANAT), which together catalyze the conversion of tryptophan into serotonin and subsequently melatonin-critical regulators of neuroendocrine balance and sleep physiology [14]. In addition, GABA present in banana peel may contribute to improved sleep quality by inhibiting excitatory neurotransmission and promoting central nervous system relaxation [15]. While previous studies have predominantly focused on the nutritional properties of banana pulp, mechanistic research regarding banana peel and its potential effects on sleep regulation remains limited, especially at the clinical level. To address this gap, the present study first analyzed the bioactive compounds present in banana peel extract (Happy Banana®) provided by TCI Co., Ltd. *In vitro* cell-based assays were then performed to investigate the extract's effects on the expression of sleep-related genes. Furthermore, a human clinical trial was conducted to evaluate its impact on sleep quality, levels of serotonin and melatonin, and autonomic nervous system balance, and subjective sleep perception assessed through self-reported questionnaires.

MATERIALS AND METHODS

Manufacturing process of banana peel extract

The banana peel extract used in this study was prepared by TCI Co., Ltd. through a standardized manufacturing process that included grinding, aqueous extraction with a food-grade acid solution, filtration, and concentration under reduced pressure. The concentrated extract was then blended with a carrier material (maltodextrin), followed by pasteurization and freeze-drying to produce a stable dosage form [16]. The final product was packaged and subjected to quality control procedures to ensure consistency and safety prior to experimental use.

Cell culture

Human neuroblastoma SH-SY5Y cells (ATCC® CRL-2266) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin (Gibco, USA), and maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Quantification of gene expressions by real-time PCR

The treated cells were harvested, and total RNA was isolated from cells using an RNA purification kit (Geneaid, Taiwan). DNA-free total RNA was reversely transcribed to cDNA using a SuperScript™ Reverse Transcriptase kit (Invitrogen, Life Technologies Co., CA, USA). Quantitative real-time PCR was conducted using an ABI Step OnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Inc., CA, USA) and the SYBR Green Master Mix (KAPA Biosystems, MA, USA) for was used transcript measurements. The reaction mixture was cycled as follows: one cycle at 95°C for 20 s, followed by 40 cycles of 95°C (1 s), 60°C (20 s), and a plate reading was conducted after each cycle. The melting curves of the PCR products were analyzed during the quantitative real-time PCR. Gene expression levels of *TPH1*, *DDC*, and *AANAT* were quantified and normalized to *GAPDH* as the internal reference gene. All reactions were performed in triplicate. The following primer sequences were used: *TPH1* (forward 5'-TGGCTTCAGCTTTGAGGATTG-3', reverse 5'-AGGAAGTGGGATGTCGTTGG-3'), *DDC* (forward 5'-TGGAGGATGCTGACTTACGG-3', reverse 5'-GGAGAGGAGGACAGGATGGA-3'), *AANAT*: (forward 5'-GCTGCTGAGGAGGTGAAAGA-3', reverse 5'-CGTTGTCCTTGTAGGCGTTC-3'); *ASMTL*: (forward 5'-ACCTTTTGGCTTTGAGGCTT-3', reverse 5'-CTTGGTGGAGGTTGAGGAGA-3'), and *GAPDH* (forward 5'-GAAGGTGAAGGTCGGAGTCA-3', reverse 5'-GAAGATGGTGATGGGATTTC-3'). Data were analyzed using

the ABI StepOne™ Software v2.2.3 (Thermo Fisher Scientific, Inc., Carlsbad, CA, USA). All PCR assays were performed in duplicate three times.

Identification of TCI-MP-14 in banana peel extract by HPLC

TCI-MP-14 (Hydroxyanigorufone), a specialized compound identified from banana peel extract, was isolated and analyzed using High-Performance Liquid Chromatography (HPLC). The banana peel extract was prepared by ethanol extraction, followed by filtration and concentration under reduced pressure. The crude extract was then reconstituted in methanol and filtered through a 0.22 μm PTFE membrane prior to HPLC analysis. HPLC was performed using a Shimadzu LC-20A system equipped with a photodiode array (PDA) detector set at 280 nm. Separation was achieved on a C18 reversed-phase column (Shim-pack XR-ODS, 150 mm × 4.6 mm, 5 μm). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), with a gradient elution program as follows: 0–5 min, 10% B; 5–25 min, linear gradient to 90% B; 25–30 min, held at 90% B. The flow rate was 1.0 mL/min, and the column temperature was maintained at 30 °C. The injection volume was 10 μL. Retention time and UV absorbance spectra of the peak corresponding to TCI-MP-14 were confirmed by comparison with a purified reference standard. The identity of TCI-MP-14 was further supported by LC-MS analysis. Quantification was based on calibration curves constructed using known concentrations of the standard compound.

Clinical trial design

The clinical study was approved by the Taipei Medical University Institutional Review Board (N202103033), and was registered on ClinicalTrials.gov Identifier: NCT05130359, and was conducted according to the code of ethics on human experimentation established by the Declaration of Helsinki (1964) and its amendments. Written informed consent was obtained from all subjects after a full explanation of the study. This study was conducted between January 2021 and December 2024, following a randomized, double-blind, placebo-controlled design. A total of 50 subjects aged 18 to 65 years meeting the Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition) criteria for insomnia disorder were enrolled. Subjects were recruited through sleep seminars or social media platforms (e.g., Facebook, LINE, Instagram). Inclusion criteria were as follows: (1) Male or female subjects aged between 18 and 65 years; (2) diagnosis of insomnia disorder, based on DSM-5 criteria, confirmed by a qualified board-certificated psychiatrist and sleep specialist; (3) a Pittsburgh Sleep Quality Index (PSQI) score greater than 5. Exclusion criteria included: (1) regular use of hypnotics or melatonin supplements within 1 month prior to the trial; (2) presence of severe psychiatric disorders; (3) pregnancy or lactation; (4) known allergy to bananas or banana peel; (5) history of cardiovascular disease; (6) a documented clinical history of severe obstructive sleep apnea. Subjects were randomly assigned to either the banana peel group (n=27), which received 2 tables of banana peel extract daily before bedtime for 4 weeks, or the placebo group (n=23). Fasting venous blood (25 mL) was collected at weeks 0, 2, and 4 to analyze routine hematological parameters (white blood cells, red blood cells, platelets), liver and kidney function markers (GOT, GPT, uric acid, BUN, creatinine), lipid profiles (cholesterol, HDL-C, LDL-C, triglycerides), serotonin. Salivary melatonin was collected both at night and upon waking. Sleep quality and duration were monitored, and self-administered questionnaires were completed as secondary outcome measures.

Supplement formulation

Banana peel group: containing Happy Banana® Banana Extract (powder) 100mg, microcrystalline cellulose, silicon dioxide, magnesium stearate. Placebo group: containing microcrystalline cellulose, silicon dioxide, magnesium stearate. Subjects took 2 tablets of the test sample two hours before bedtime each day for 4 consecutive weeks. The placebo, banana peel group were packaged in the same appearance, shape, and size.

Salivary melatonin measurement

Salivary melatonin concentrations were measured to evaluate changes in circadian rhythm in response to the intervention. Saliva samples were self-collected at two specific time points: (1) at night, within 30 minutes before bedtime, and (2) in the morning, immediately upon waking and prior to any food or drink intake. Collections were performed at three study time points: baseline before product intake, after 2 weeks of continuous use, and after 4 weeks. All samples were immediately stored at -20°C after collection and transported to the laboratory under temperature-controlled conditions. Melatonin concentrations were quantified using the Salivary Melatonin ELISA Kit (Salimetrics, LLC, State College, PA, USA), a competitive immunoassay specifically designed for the quantitative determination of melatonin in saliva.

Sleep quality examination

This study utilized the sleep quality examination system developed by LARGAN Health AI-TECH Co., LTD (Taichung City, Taiwan) to assess sleep parameters. This device is a portable medical instrument approved by the Taiwan Ministry of Health and Welfare (License No. MOHW Medical Devices Manufacturing No. 007003) and is suitable for home use [17]. The system employs a wearable Holter Electrocardiogram (ECG) device that records ECG and Accelerometer (ACC) signals during sleep. The ACC detects body movements and sleep posture changes, while the ECG analyzes sinus heart rate and respiration-related activity. By integrating data from both ECG and ACC, the system evaluates sleep quality and measures heart rate and Heart Rate Variability (HRV). In addition, the system incorporates Cardio Pulmonary Coupling (CPC) analysis to provide a more comprehensive and reliable evaluation of sleep parameters. Due to its user-friendly design and minimal interference with sleep, it is particularly well-suited for home-based monitoring. Prior to the assessment, each subject received one-on-one instruction from a trained operator on how to properly use the device. Instructional materials and a 24-hour contact number were also provided to assist with

troubleshooting during usage. Each subject was instructed to take two tablets of the investigational product two hours before bedtime. They were then required to wear the ECG device before sleep and remain seated calmly on the bed for five minutes without using a mobile phone or engaging in any other activity. After the five-minute resting period, subjects could proceed to sleep while wearing the device. The device setup was as follows: the subject pressed the button on top of the ECG monitor. A green or blue light indicated that the device was functioning properly, whereas a red or yellow light signaled the need for replacement. Three adhesive electrodes were attached to the device, the backing papers were removed, and the device was affixed to the chest near the heart. Two surgical tapes were applied in a crisscross manner to ensure secure attachment. Upon waking, subjects were instructed to immediately remove the device and return it to the study site, where it was collected and processed by the research operator. All recorded data were automatically uploaded *via* a wireless network to a cloud-based platform for analysis.

Statistical analysis

The comparison of measurement results for blood test data, sleep monitoring reports, and other related parameters among groups and between groups was analyzed by student's t-test through GraphPad Prism, as $p<0.05$ was considered statistical significance.

RESULTS

Identification and functional evaluation of bioactive compounds in banana peel extract for sleep-related pathways *in vitro*

This study aimed to investigate the potential regulatory effects of bioactive compounds derived from banana peel extract on sleep-related metabolic pathways, with a particular focus on the biosynthetic conversion of tryptophan into serotonin and melatonin. Based on the analysis of bioactive components, a physiologically active compound, Hydroxyanigorufone, was identified in banana peel extract and selected as the primary substance for further investigation (Figure 1A). Gene expression analysis was conducted in cells treated with Hydroxyanigorufone, revealing that banana peel extract significantly modulated key genes involved in the tryptophan metabolic pathway, including TPH1 (Tryptophan Hydroxylase 1) with a 4.09-fold increase ($p<0.01$), DDC (Dopa Decarboxylase) with a 1.57-fold increase (not significant), AANAT (Arylalkylamine N-acetyltransferase) with a 2.82-fold increase ($p<0.01$), and ASMT (Acetylserotonin O-methyltransferase) with a 2.37-fold increase ($p<0.05$) (Figure 1B). Likewise, cells treated with banana peel extract showed a consistent pattern of gene activation: TPH1 increased by 1.98-fold, DDC by 2.29-fold, and AANAT by 3.33-fold ($p<0.01$) (Figure 1C). These findings suggested that Hydroxyanigorufone may enhance the biosynthesis of serotonin and melatonin by upregulating the expression of key enzymes involved in the tryptophan metabolic pathway, indicating its potential for promoting physiological relaxation and improving sleep (Figure 1).

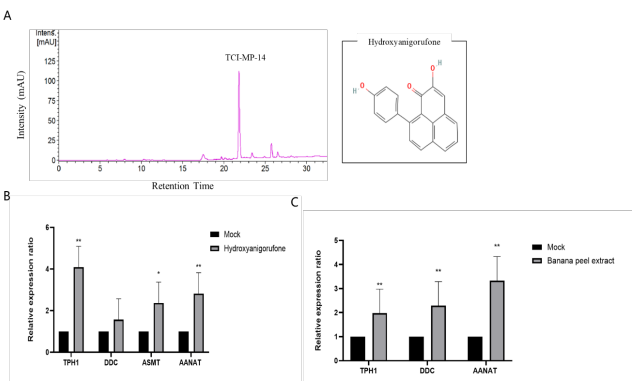

Figure 1. Identification and *in vitro* functional analysis of Hydroxyanigorufone from banana peel extract. (A) HPLC chromatogram showing the major peak identified as Hydroxyanigorufone in banana peel extract and its corresponding chemical structure. (B) Gene expression analysis in cultured cells treated with Hydroxyanigorufone for 24 hours. mRNA levels of key enzymes in the tryptophan metabolic pathway TPH1 (Tryptophan Hydroxylase 1), DDC (Dopa Decarboxylase), AANAT (Arylalkylamine N-acetyltransferase), and ASMT (Acetylserotonin O-methyltransferase) were analyzed by qPCR. (C) Relative expression of TPH1, DDC, and AANAT in cells treated with whole banana peel extract under the same conditions. All data are presented as mean ± SD from three independent experiments. *$p<0.05$, **$p<0.01$ compared to the mock control.

Banana peel extract enhanced sleep physiology *via* neuroendocrine modulation in human

Based on the above in vitro results indicating the potential of banana peel extract to modulate the tryptophan metabolic pathway, a human clinical trial was subsequently designed to verify its regulatory effects on sleep-related physiological markers *in vivo*. Table 1 presents the baseline characteristics of the study subjects. The heart rate variability analysis was used to assess autonomic nervous system balance, with the LF/HF ratio (low-frequency to high-frequency power; sympathetic to parasympathetic ratio) serving as an index of sympathetic–parasympathetic activity. Compared to the placebo group, subjects who consumed banana peel extract for 4 consecutive weeks exhibited a significant reduction in LF/HF ratio, reaching -0.85 at week 2 and maintaining a level of -0.91 at week 4 ($p<0.05$), whereas the placebo group showed a progressive increase, reaching +1.53 (Figure 2A). As for other objective sleep parameters in Table 2, including Sleep Onset Latency (SOL), Wake After Sleep Onset (WASO), Total Sleep Time (TST), light sleep, deep sleep, and Rapid Eye Movement (REM) sleep assessed by CPC analysis, no statistically significant differences were observed between the banana peel extract group and the placebo group. The above results suggested that banana peel extract may exert beneficial physiological effects during sleep by modulating the autonomic nervous system.

Table 1. Baseline characteristics of the study subjects.

	Banana peel group (n=27)	Placebo group (n=23)
Age (years)	33.04 ± 10.12	32.70 ± 6.86
Gender		
Male	11	10
Female	16	13
BMI (kg/m2)	22.98 ± 4.07	21.51 ± 2.96

Table 2. Physiological sleep parameters.

Items	Banana peel group (n=27)	Placebo group (n=23)	p value
Sleep Onset Latency (SOL), min	mean (SD)		
week 0	14.6 (17.4)	12.4 (10.1)	0.58
week 2	13.4 (12.8)	10.0 (7.6)	0.24
week 4	14.9 (13.3)	10.7 (9.2)	0.2
Wake after sleep onset (WASO), min			
week 0	15.8 (12.8)	16.4 (8.1)	0.84
week 2	21.7 (16.4)	19.7 (14.5)	0.65
week 4	22.1 (16.5)	23.7 (20.4)	0.75
Total sleep time (TST), min			
week 0	369.9 (51.2)	370.9 (56.4)	0.94
week 2	365.4 (56.4)	363.0 (57.0)	0.88
week 4	369.8 (69.8)	376.6 (55.2)	0.7
Light Sleep, %			
week 0	37.4 (14.0)	33.6 (8.9)	0.25
week 2	36.5 (12.2)	28.7 (9.6)	0.07
week 4	36.6 (12.8)	32.6 (8.8)	0.21
Deep sleep, %			
week 0	39.0 (12.3)	45.7 (16.8)	0.11
week 2	38.6 (15.4)	45.5 (14.3)	0.1
week 4	39.1 (15.6)	42.3 (14.7)	0.45
REM Sleep, %			
week 0	19.0 (7.7)	20.7 (7.2)	0.42

week 2	19.1 (7.9)	20.6 (6.7)	0.48
week 4	18.3 (8.9)	19.2 (6.8)	0.69
Sympathetic to parasympathetic ratio (LF/HF)			
week 0	3.3 (4.0)	1.8 (1.4)	0.07
week 2	2.5 (2.2)	2.3 (1.7)	0.79
week 4	2.4 (1.9)	3.3 (4.0)	0.3

In addition, serum serotonin levels were measured to further evaluate the neuromodulatory potential of banana peel extract. Compared to the baseline (Week 0), subjects who consumed banana peel extract for 2 and 4 weeks showed increases in serum serotonin levels of 5.7% and 13.9%, respectively, with the change at week 4 reaching statistical significance ($p<0.05$)(Figure 2B). Salivary melatonin levels were also evaluated to assess the downstream impact of increased serotonin synthesis on circadian rhythm regulation. Compared to baseline, subjects who consumed banana peel extract showed a 24.3% increase in melatonin concentration at week 2 and a 43.3% increase at week 4. The salivary melatonin levels in the banana peel extract group were significantly higher than those in the placebo group at both time points ($p<0.05$) (Figure 2C). To further assess circadian rhythm regulation, the difference in melatonin concentration between nighttime and daytime (night–day delta) was also analyzed. At baseline, subjects in the banana peel extract group exhibited a negative nighttime–daytime melatonin differential (-5.5 pg/mL), indicating a blunted melatonin secretion profile. After 2 and 4 weeks of supplementation, this differential increased by 133% and 209%, respectively (Figure 2D), indicating a pronounced elevation in nocturnal melatonin secretion relative to daytime levels. This shift reflects an improvement in circadian alignment and suggests that banana peel extract may promote a more physiologically appropriate melatonin rhythm conducive to sleep (Figure 2).

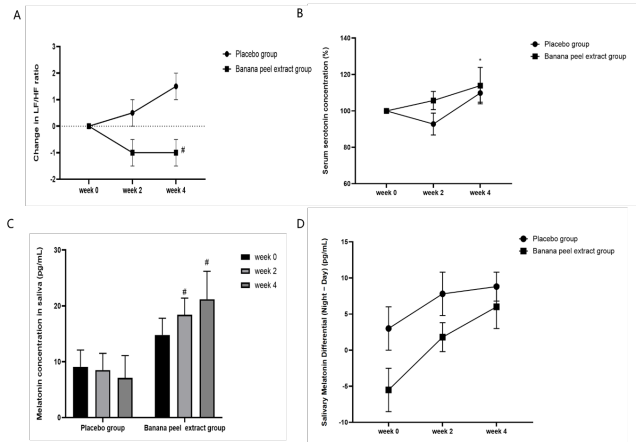

Figure 2. Effects of banana peel extract on sleep-related physiological markers in human subjects. Subjects were randomly assigned to either the placebo group or the banana peel extract group. Each subject took two tablets of the test product daily (total dose: 200 mg/day) two hours before bedtime. Sleep-related physiological and neuroendocrine parameters were evaluated at weeks 0, 2, and 4, including (A) heart rate variability (LF/HF ratio), (B) serum serotonin concentration, (C) salivary melatonin concentration, and (D) the night-day differential in salivary melatonin levels. Compared to the placebo group: #, $p<0.05$. Compared to baseline (Week 0): * $p<0.05$.

Banana peel extract improved subjective sleep quality without adverse effects

To assess subjective improvements in daytime alertness and sleep continuity, questionnaire data were collected at baseline, week 2, and week 4. Compared to baseline, the frequency of daytime drowsiness in the banana peel extract group decreased by 10.2%at week 2 and 13.9% at week 4, suggesting improved daytime vitality (Figure 3A). Additionally, the frequency of nocturnal urination decreased by 12.7% and 19.0% at weeks 2 and 4, respectively, indicating enhanced sleep continuity and reduced nighttime arousal (Figure 3B). Safety was further evaluated *via* routine hematological and biochemical blood tests. As shown in Table 3, supplementation with banana peel extract for 4 weeks had no adverse effects on White Blood Cell (WBC) count, Red Blood Cell (RBC) count, hemoglobin, hematocrit, or platelet count. Similarly, no significant changes were observed in liver function markers (ALT, AST), kidney function markers (BUN, creatinine, uric acid), or blood lipids (cholesterol, triglycerides), indicating good overall tolerability and systemic safety (Figure 3).

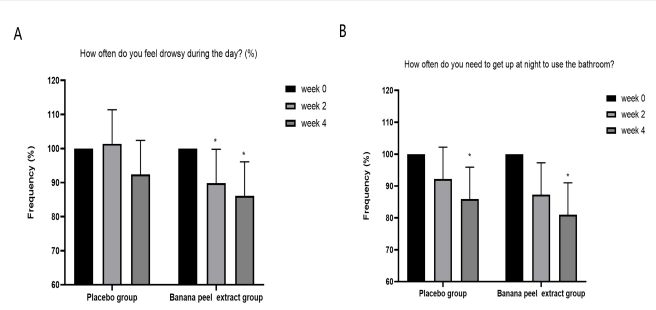

Figure 3. Effects of banana peel extract on subjective sleep-related symptoms. Subjects were randomly assigned to either the placebo group or the banana peel extract group. Each subject took two tablets of the investigational product two hours before bedtime daily. Subjective sleep quality was assessed *via* questionnaire at Week 0, Week 2, and Week 4, including evaluations of (A) Frequency of daytime drowsiness and (B) Frequency of nocturnal urination events. Compared to baseline (Week 0): * $p<0.05$.

Table 3. Routine blood test evaluation.

Items	Banana peel group (n=27)	Placebo group (n=23)	p value
WBC (10X3/uL)			
week 0	6.3 (1.4)	5.9 (2.2)	0.59
week 2	6.3 (1.7)	5.5 (1.4)	0.13
week 4	6.2 (1.6)	6.1 (1.7)	0.81
Neutrophils (%)			
week 0	54.9 (10.0)	55.7 (8.1)	0.77
week 2	56.5 (9.0)	54.4 (7.5)	0.41
week 4	54.8 (8.7)	57.0 (10.5)	0.47
Lymphocytes (%)			
week 0	32.4 (7.7)	32.9 (7.6)	0.85
week 2	32.1 (8.5)	33.7 (7.4)	0.52
week 4	32.6 (8.1)	32.1 (9.9)	0.85
Monocytes (%)			
week 0	7.7 (2.1)	7.7 (1.9)	0.97
week 2	7.7 (1.7)	7.9 (2.0)	0.63
week 4	8.4 (3.1)	7.5 (2.1)	0.3
Eosinophils (%)			
week 0	2.8 (1.5)	3.1 (2.1)	0.71
week 2	3.0 (1.6)	3.2 (2.5)	0.69
week 4	3.5 (2.3)	2.8 (2.1)	0.33
Basophils (%)			
week 0	0.8 (0.4)	0.7 (0.3)	0.45
week 2	0.7 (0.4)	0.7 (0.3)	0.99
week 4	0.7 (0.4)	0.6 (0.3)	0.33
RBC (10^6/uL)			
week 0	4.7 (0.6)	4.9 (0.6)	0.34
week 2	4.7 (0.6)	4.8 (0.6)	0.47
week 4	4.7 (0.6)	4.9 (0.6)	0.38

Hemoglobin (g/dL)			
week 0	13.9 (1.3)	13.6 (1.3)	0.44
week 2	13.8 (1.2)	13.5 (1.4)	0.39
week 4	13.9 (1.2)	13.6 (1.6)	0.54
Hematocrit (%)			
week 0	41.2 (3.8)	40.7 (3.7)	0.67
week 2	41.2 (3.4)	40.3 (3.8)	0.43
week 4	41.3 (3.6)	40.8 (4.3)	0.68
M.C.V (fL)			
week 0	88.3 (6.2)	80.6 (18.7)	0.08
week 2	88.5 (6.4)	84.4 (8.8)	0.09
week 4	88.4 (6.1)	84.6 (8.9)	0.1
M.C.H (pg)			
week 0	29.8 (2.4)	28.2 (3.5)	0.09
week 2	29.7 (2.5)	28.3 (3.4)	0.11
week 4	29.8 (2.4)	28.3 (3.4)	0.1
M.C.H.C (g/dL)			
week 0	33.7 (0.6)	33.3 (0.9)	0.14
week 2	33.6 (0.6)	33.4 (0.8)	0.51
week 4	33.7 (0.7)	33.4 (0.8)	0.25
Platelet count (10^3/uL)			
week 0	254.0 (103)	248.5 (55.2)	0.83
week 2	261.1 (82.5)	252.7 (47.0)	0.66
week 4	253.6 (89.0)	254 (52.0)	0.99
ALT (U/L)			
week 0	16.4 (13.7)	16.9 (13.9)	0.91
week 2	20.2 (23.7)	22.6 (29.6)	0.78
week 4	16.3 (12.6)	24.0 (21.9)	0.18
AST (U/L)			
week 0	18.0 (7.7)	18.3 (4.9)	0.88
week 2	22.1 (22.8)	21.4 (18.9)	0.92

week 4	18.3 (6.2)	19.7 (7.2)	0.53
BUN (mg/dL)			
week 0	12.6 (2.7)	12.7 (2.5)	0.87
week 2	12.1 (2.4)	12.6 (3.7)	0.61
week 4	12.5 (2.7)	12.9 (3.8)	0.68
Creatinine (mg/dL)			
week 0	0.7 (0.2)	0.7 (0.1)	0.62
week 2	0.7 (0.2)	0.7 (0.2)	0.78
week 4	0.7 (0.2)	0.7 (0.2)	0.95
Uric acid (mg/dL)			
week 0	5.6 (1.6)	5.2 (1.5)	0.36
week 2	5.8 (1.6)	5.2 (1.4)	0.22
week 4	5.5 (1.5)	5.2 (1.4)	0.46
Cholesterol (mg/dL)			
week 0	180.7 (30.0)	183.6 (29.3)	0.75
week 2	179.5 (25.5)	182.2 (26.7)	0.74
week 4	176.8 (30.3)	192.2 (36.6)	0.14
HDL-C (mg/dL)			
week 0	62.4 (15.0)	66.6 (16.9)	0.4
week 2	63.9 (16.7)	67.4 (17.0)	0.5
week 4	61.5 (13.9)	67.8 (13.4)	0.14
LDL-C (mg/dL)			
week 0	105.7 (29.1)	106.3 (28.5)	0.95
week 2	103.2 (25.7)	104.1 (27.7)	0.91
week 4	102.2 (26.4)	114.5 (39.4)	0.24
Triglyceride (mg/dL)			
week 0	84.3 (50.8)	86.2 (71.9)	0.92
week 2	103.4 (66.6)	75.7 (36.0)	0.1
week 4	101.2 (64.1)	80.1 (55.4)	0.26

DISCUSSION

Banana peels, a major by-product of banana processing, have long been regarded as agricultural waste. Their disposal not only incurs costs but may also pose environmental burdens. However, recent investigations into their phytochemical composition and functional properties have revealed their high-value potential as

a natural resource [18]. Banana peels are rich in polyphenols, flavonoids, amine compounds, and dietary fiber, and exhibit notable antioxidant and anti-inflammatory activities [19]. In this study, a physiologically active compound, Hydroxyanigorufone, was identified from banana peel extract. *In vitro* cell experiments demonstrated that Hydroxyanigorufone significantly upregulated the expression of key enzymes involved in the tryptophan metabolic pathway namely TPH1, AANAT, and ASMT, thereby enhancing the biosynthetic potential for serotonin and melatonin. Subsequent human clinical trials further confirmed that four weeks of continuous consumption of banana peel extract resulted in significant improvements in autonomic nervous system balance (as evidenced by reduced LF/HF ratio), elevated serum serotonin and salivary melatonin concentrations, and improved circadian rhythm alignment (as shown by increased nighttime–daytime melatonin differentials). Subjects also reported decreased daytime drowsiness and reduced nighttime awakenings in subjective questionnaires, indicating potential benefits for sleep promotion, emotional stabilization, and enhanced daytime vitality. Additionally, no abnormal changes were observed in hematological and biochemical parameters, supporting the extract's safety and tolerability. These findings not only highlight the functional potential of banana peels but also underscore their applicability as a sustainable resource for the development of health-promoting products.

Bananas (*Musa spp.*) and their peels have traditionally been regarded as sleep-promoting foods in many cultures [20]. Their sleep-enhancing mechanisms have been partially attributed to their high tryptophan content a vital amino acid and biochemical precursor to both serotonin and melatonin [13]. Tryptophan is first converted to 5-HTP *via* TPH, then decarboxylated by DDC to form serotonin [21]. Subsequently, serotonin is transformed into melatonin through the sequential actions of AANAT and ASMT [22]. Beyond tryptophan, banana peels are rich in a variety of bioactive compounds, including GABA, flavonoids, and polyphenols. Prior phytochemical analyses of banana peels have identified numerous functional constituents, such as catecholamines, flavonols, and phenolic acids [23]. This study specifically focused on Hydroxyanigorufone, a novel physiologically active compound derived from banana peel extract, which exhibited significant potential to modulate the expression of genes associated with tryptophan metabolism. *In vitro* results showed that Hydroxyanigorufone markedly enhanced the expression of TPH1, AANAT, and ASMT, suggesting its capacity to facilitate serotonin and melatonin biosynthesis. Although current study on Hydroxyanigorufone remains limited, its chemical structure bears resemblance to known phenolic compounds with antioxidant and neuroactive properties, indicating its potential neuromodulatory function.

Although this study found that banana peel extract did not produce statistically significant differences in objective sleep parameters assessed by CPC analysis such as SOL, WASO, TST, light sleep, deep sleep, and REM sleep it demonstrated significant improvements in autonomic nervous system balance (as indicated by a reduced LF/HF ratio) and neuroendocrine markers (increased serum serotonin and salivary melatonin levels). These findings suggest that the potential sleep-promoting mechanism of banana peel extract may be closely related to the modulation of the pre-sleep readiness state, rather than a direct alteration of objective sleep parameters in current 4-week clinical trial [24]. This effect may reflect the extract's regulatory capacity on psychological relaxation, stress reduction, and circadian rhythm alignment, thereby facilitating more stable conditions for sleep initiation. The proposed mechanisms may involve: (1) suppression of excessive sympathetic activity and enhancement of parasympathetic tone, resulting in improved HRV and a more relaxed physiological state [25]; and (2) upregulation of key enzymes in the tryptophan metabolic pathway TPH1, AANAT, and ASMT leading to enhanced conversion of serotonin into melatonin and contributing to a circadian peak in melatonin secretion prior to sleep onset [26]. This is further supported by the observed increase in the nighttime–daytime differential of salivary melatonin levels.

Such mechanisms have also been observed in various plant-derived compounds. For instance, clinical studies on *Valeriana officinalis* (valerian) at daily doses of 400–900 mg have demonstrated significant reductions in SOL and improvements in subjective sleep quality, with mechanisms attributed to its affinity for GABA-A receptors and its role in enhancing parasympathetic activity [27]. *Passiflora incarnata* (passionflower), administered at a daily dose of 500 mg, has been shown to reduce cortisol responses and anxiety scores, thereby mitigating sleep disturbances. Its active constituents, including isoflavones and coumarins, are believed to enhance GABAergic activity [28]. *Melissa officinalis* (lemon balm) and *Matricaria chamomilla* (chamomile) contain rosmarinic acid and apigenin, respectively [29]. Rosmarinic acid inhibits GABA transaminase, while apigenin directly binds to GABA-A receptors [30]. Human trials have reported significant improvements in stress-related and anxiety-associated insomnia within two weeks of supplementation [30]. *Crocus sativus* (saffron), whose active components safranal and crocin exhibit serotonin reuptake inhibition activity, has been validated in multiple randomized, double-blind clinical trials at daily doses of 28-30 mg [31]. These studies confirmed improvements in PSQI scores, SOL, and both subjective and objective sleep parameters. Similar to these plant-based interventions, banana peel extract demonstrated beneficial effects on neuroendocrine regulation, including significant increases in serum serotonin and salivary melatonin levels, along with improvements in circadian rhythm alignment and autonomic nervous system balance. While objective sleep parameters such as SOL and TST did not show significant changes, the neurophysiological markers observed suggest that banana peel extract, like *Melissa officinalis, Matricaria chamomilla* and *Crocus sativus*, may exert sleep-promoting effects through indirect modulation of stress and arousal pathways. It is reasonable to assume that extending the trial period may lead to more pronounced effects on objective sleep parameters. These converging lines of evidence support the role of banana peel extract as a natural agent for improving sleep readiness and enhancing sleep-related neurochemical profiles, warranting further investigation alongside established phytotherapeutics. The present study had several limitations. First, although Hydroxyanigorufone was

identified as a key active compound and shown *in vitro* to enhance tryptophan metabolism-related gene expression, it was not validated in human trials. Therefore, its *in vivo* effects remained unclear. Second, the study involved a limited sample size focused on adults with sleep disturbances. Further studies with larger and more diverse populations were needed.

CONCLUSION

This study systematically validated the sleep-promoting potential of banana peel extract (Happy Banana®) through both *in vitro* and human clinical investigations. The findings revealed that the extract may enhance the biosynthesis of serotonin and melatonin, thereby modulating the autonomic nervous system and optimizing physiological readiness for sleep. As a major byproduct of banana processing, banana peel has long been regarded as agricultural waste. However, it is rich in polyphenols, flavonoids, amines, and dietary fiber functional constituents with growing scientific interest. Notably, this study identified Hydroxyanigorufone, a physiologically active compound within the extract, further supporting its development value. Future directions may include sustainable agricultural waste reutilization, development of functional foods, formulation of plant-based synergistic blends, and personalized sleep nutrition solutions.

Credit authorship contribution statement

Hsin-Chien Lee, Chi-Fu Chiang: Data curation, Investigation, Methodology, Writing-original draft. Hsin-Chien Lee: Formal analysis, Software, Writing-review and editing. Yung-Hsiang Lin: Resources. Lee, Hsin-Chien: Conceptualization, Funding acquisition, Project administration, Supervision, Writing–review and editing. Shu-Ting Chan, Yung-Kai Lin, Chi-Fu Chiang: Conceptualization, Funding acquisition, Project administration, Writing-review and editing.

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