Iranian Journal of Pharmaceutical Sciences
2024: 20 (4): 336- 344
https://journals.sbmu.ac.ir/IJPS

Original Article

Detection and Quantification of GABA and Melatonin Contents in Five Hypnotic Medicinal Plants using Chromatography-Based Techniques

Marjan Talebi[a,b], Malak Obeid[a], Faraz Mojab[b,c*]

[a]*Student Research Committee, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [b]*Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [c]*Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Abstract

Over the years, medicinal plants have been considered promising treatments for managing various disorders. Numerous natural products contain prospective potential for the treatment of insomnia. This study aimed to determine the quantities of melatonin and GABA present in five plant extracts with hypnotic effects. Five plants were selected for investigation, including *Valeriana officinalis*, *Passiflora incarnata*, *Aloysia citriodora*, *Withania somnifera*, and *Lavandula officinalis*. The ethanolic extracts of these plants were prepared using the maceration method. For preliminary phytochemical evaluations, HPTLC fingerprinting was conducted on both the extracts and the standards, including melatonin and GABA. GABA content was determined using HPTLC in three out of the five plants. Furthermore, the HPLC method was used to confirm the presence of melatonin. The results of HPTLC fingerprinting revealed that the ethanolic extracts of *Aloysia citriodora*, *Passiflora incarnata*, and *Withania somnifera* contained GABA, which could be attributed to their reported hypnotic effects. Conversely, none of the five plants contained melatonin, indicating that the hypnotic effect was not related to this compound. In conclusion, this study described the HPTLC method for detecting and quantifying GABA in *Withania somnifera* and *Aloysia citriodora* for the first time. Notably, *Withania somnifera* exhibited the highest amount of GABA.

Keywords: Chromatography, GABA, HPLC, HPTLC, Hypnotic, Melatonin, Quantitative analysis.

1. Introduction

Insomnia is a prevalent and spreading disorder, especially in the elderly. Several research studies have reported that insomnia affects approximately 12-20% of the worldwide population [1, 2]. The US FDA has approved benzodiazepines, non-benzodiazepines, H_1-receptor antagonists, melatonin agonists, and orexin antagonists as promising therapeutic agents for insomnia management. Moreover, particular antidepressants, such as amitriptyline and trazodone, alongside anticonvulsant medications like barbiturates, gabapentin, and pregabalin, have been used as therapeutic

Corresponding Author: Faraz Mojab (Pharm.D., Ph.D.), Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: sfmojab@sbmu.ac.ir

Cite this article as: Talebi M, Obeid M, Mojab F. *Detection and Quantification of GABA and Melatonin Contents in Five Hypnotic Medicinal Plants using Chromatography-Based Techniques*. Iran. J. Pharm. Sci., 2024, 20 (4): 336- 344.

DOI: https://doi.org/10.22037/ijps.v20i4.45480

interventions for the condition of insomnia [3–5]. Despite the existence of acceptable therapeutic outcomes with the use of pharmacotherapeutics for insomnia, patients persist in expressing their displeasure regarding the concurrent unwanted effects, including impaired cognitive function, physical dependence, and lethargy [6, 7].

In order to mitigate the previously mentioned displeasure, it is conceivable to integrate natural substances as an alternative treatment. In recent times, a multitude of medicinal plants have undergone consideration to investigate their sedative properties [8]. *Aloysia citriodora*, *Coriandrum sativum*, *Crocus sativus*, *Dimocarpus longan*, *Humulus lupulus*, *Lavandula officinalis*, *Matricaria chamomilla*, *Melissa officinalis*, *Passiflora incarnata*, *Rhus parviflora*, *Rosa damascena*, *Sesamum indicum*, *Stachys lavandulifolia*, *Valeriana officinalis*, and *Zizyphus jujuba* are some of the medicinal plants that have been used to ameliorate sleep disorders [9– 12].

Melatonin is derived from tryptophan, a ubiquitous indolamine found in bacteria, algae, fungi, plants (e.g., tomato and tobacco), insects, and vertebrates, including humans [13, 14]. The activity of this system changes during the day and night depending on the light [15]. Melatonin and its analogs interact with melatonin receptors in the body and play an important role in the management of various disorders, including insomnia, Alzheimer's disease, depression, epilepsy, migraine, cancer, cardiovascular disorders, obesity, diabetes, and disorders of the immune system [16, 17].

GABA is a non-protein four-carbon amino acid in plants, bacteria, the brain, and other animal tissues [18–20]. GABA is produced when glutamic acid is decarboxylated and catalyzed by the enzyme glutamate decarboxylase [21]. In mammals, GABA exhibits a wide range of health benefits related to promoting sleep quality, alleviating mental and physical stress, boosting the immune system under challenging conditions, slowing down aging symptoms, strengthening blood vessels, and addressing various neurological disorders. The presence of glutamate and GABA has been detected in various species of plants. Cereals, vegetables, fruits, and specific herbs such as chamomile, sage, and lavender are the most abundant sources of these amino acids [22].

Aloysia citriodora traditionally used as a sedative, anticonvulsant, diuretic, heart palpitation and dizziness reliever [23,24]. Some of the neuroprotective effects of *Aloysia citriodora* are related to the interaction with the benzodiazepine site of the GABA receptors [25]. *Valeriana officinalis* roots are used in traditional medicine as a sedative-hypnotic medicinal plant. One of the proven sleep-inducing mechanisms of valerian is the stimulation of $GABA_A$ by valeric acid [26,27] *Passiflora incarnata* leaves and flowers have a mild smell and are recommended to reduce stress and insomnia caused by them [28]. Studies conducted *in vivo* on *Withania somnifera* root extract have shown that this plant controls insomnia and related behavioral and biochemical changes [29][30]. *Lavandula officinalis* exhibits notable neuroprotective effects such as anticonvulsive, sedative-hypnotic, and antidepressant effects [31].

This study prepared ethanolic extracts of *Valeriana officinalis*, *Passiflora incarnata*, *Aloysia citriodora*, *Withania somnifera*, and *Lavandula officinalis* for analysis. The HPTLC fingerprinting was implemented on extracts and GABA to determine and quantify GABA content. Furthermore, the presence of melatonin in the plant extracts was investigated using the HPLC method.

2. Materials and Methods

2.1. Chemicals and Reagents

All solvents, chemicals, and reagents used for extraction and chromatographic analysis possessed the requisite degree of purity and were purchased from Merck (Darmstadt, Germany).

2.2. Plant identification and Extraction

The plants were purchased from a local market in Tehran, Iran, except for the valerian provided by Zardband Pharmaceuticals (Tehran, Iran) with batch no. #4012-001 in the spring of 2022. F. Mojab identified the plants. The voucher specimens for *Lavandula officinalis* (SBMU-1082) and *Aloysia citriodora* (SBMU-8290) were deposited in the herbarium of the Pharmacognosy Department, SBMU Pharmacy School. Additionally, the voucher specimens for *Valeriana officinalis* (HPSRC-102), *Passiflora incarnata* (HPSRC-103), and *Withania somnifera* (HPSRC-104) were deposited in the herbarium of the Pharmaceutical Sciences Research Center, SBMU.

The ground-dried plants were macerated by ethanol (96%) to prepare extracts. Subsequently, the extracts were filtered through Whatman paper and concentrated using a Heidolph rotary evaporator (Schwabach, Germany) at 50 °C and 80 rpm. The resulting dried extracts were then stored in a freezer at -20°C until the commencement of the subsequent experiments.

2.3. Preparation of Various Concentrations of Plants Extracts

A total of 100 mg of each extract was weighed and diluted with deionized water and methanol (1:1) in a 100 ml flask, and the initial stock was made with a concentration of 1 mg/ml to prepare the initial stock for the extracts. Moving forward, dilutions were made to obtain concentrations of 2.5, 5, 10, 25, 50, 100, 250, and 500 µg/ml.

2.4. Preparation of Standard Solutions

To prepare the primary melatonin solution, 100 mg of melatonin powder was dissolved with 8 ml of methanol using a Shimadzu ultrasonic bath (Kyoto, Kyoto Prefecture, Japan). Then, the solution was filtrated using a 0.22 µm syringe filter and made up to volume in a volumetric flask with deionized water and methanol (1:1). The obtained concentration was 1 mg/ml. In the next step, the serial dilution method made concentrations of 1, 5, 10, 25, 50, 100, 200, 400, and 800 (g/ml). The melatonin calibration curve was created using the HPLC-UV device in the 10-200 µg/ml range. Melatonin concentration was calculated based on HPLC signals. 100 mg/ml Concentrations were prepared with GABA, and the initial amount of the extract was dissolved in deionized water and methanol (0.6:0.4).

2.5. Analysis of Extracts and Melatonin by TLC

Aluminum TLC plates coated with silica gel with a height of 9 cm and mobile phase MeOH-$CHCl_3$ were used. A concentration of 800 µg/ml

of each extract and 50 µg/ml of melatonin was solved in methanol. After chromatography was performed and the plates were dried, ninhydrin reagent was used. The spots appeared as pale violet bands, and the R_f of the spots was determined.

2.6. Analysis of Extracts and Melatonin by HPLC

HPLC analysis of the extracts and melatonin was performed using a Shimadzu SCL-10AVP HPLC system (Kyoto, Kyoto Prefecture, Japan). This instrument consists of an LC-10ADVP micro-piston pump, column oven, four-channel membrane degassers (DGU-14A), and Diode array detector (DAD) (SPD-10AVP, 190-600 nm). Samples were loaded on a Macherey-Nagel nucleoside-100 C18 column (250 mm × 4.6 mm, five µm; Düren, Nordrhein-Westfalen, Germany) using gradient elution and a binary mobile phase with a flow rate of 1 µl/min, column temperature at 35 °C, and a sample injection volume of 10 µl. The gradient program was as follows: A) Mobile phase: acetonitrile+phosphate buffer at pH=4.5, B) Mobile phase composition: methanol+phosphoric acid+deionized water at pH=3.

The gradient program for melatonin detection and quantification in the mentioned plants was as follows: 0-5 min, 0-5% B; 5-10 min, 5-20% B; 10-30 min, 20-50% B; 30-50, 50-100% B. The total run time was 50 minutes, and a detection wavelength of 280 nm was employed [16, 32, 33].

2.7. Analysis of Extracts and GABA by TLC

For this purpose, the mobile phase of butanol, acetic acid, and water was prepared in a ratio of 5:2:2. Extracts with a concentration of 100 mg/ml and standard GABA solution with a concentration of 10 mg/ml were loaded on the TLC plate. The plate was placed in the aforementioned mobile phase in the next step. After performing chromatography and spraying the reagent, spots appeared.

2.8. Quantification of GABA Contents in Extracts by HPTLC

The amount of GABA in the extract of *Passiflora incarnata*, *Aloysia citriodora*, and *Withania somnifera* was determined by a Camag HPTLC system (Berlin, Germany).

The stationary phase of HPTLC was silica gel, and the mobile phase was butanol, water, and acetic acid. Nihydrin was used as a reagent. The samples were prepared manually, the machine automatically did the staining, and the peaks were observed at a UV wavelength of 550 nm.

3. Results and Discussion

3.1. Yield of Plant Extraction

The yield of plant extraction using the maceration method and ethanol solvent is represented in **Table 1**.

Table 1. Yield of extraction**.**

Plant	Yield of extraction (%)
Valeriana officinalis	9.5
Passiflora incarnata	9.7
Aloysia citriodora	9.9
Withania somnifera	9.2
Lavandula officinalis	9.1

3.2. Results of TLC Fingerprinting and HPLC analysis of Melatonin and Plants

The preliminary investigation of plant extracts and Melatonin by TLC led to the observation of extremely light and narrow spots in the area, similar to *Withania somnifera* and *Valeriana officinalis*. However, HPLC analysis did not detect melatonin in plant samples. Therefore, it was inferred that the TLC spots might be attributed to an indoleamine but not melatonin.

3.3. HPTLC Fingerprinting of GABA and Plants

The preliminary investigation of plant extracts and GABA by TLC showed the presence of GABA in *Passiflora incarnata*, *Aloysia citriodora*, and *Withania somnifera*. R_f for GABA was equal to 0.55. Afterward, the HPTLC was performed following the reagent spraying, and the spots were visualized under UV at 550 nm **(Figure 1)**.

3.4. Quantification of GABA by HPTLC

The 3D-Chromatograms of GABA and samples are shown in **Figure 2**. The calibration curve for GABA **(Figure 3)** was provided by considering the peak areas in HPTLC analysis. The GABA content was calculated as 0.495, 0.475, and 0.214 mg/ml for *Withania somnifera*, *Passiflora incarnata*, and *Aloysia citriodora*, respectively **(Table 2)**. The highest GABA content was detected and quantified in the *Withania somnifera* ethanolic extract.

In recent years, due to the negative effects of synthetic hypnotic drugs, melatonin has become very important [5,7]. Medicinal plants can act in the progress of the pharmaceutical industry and the development of drugs that have great value [34].

Table 2. GABA content in plant samples was calculated based on cure areas**.**

Plant extract	GABA content (mg/ml)
Aloysia citriodora	0.214
Withania somnifera	0.495
Passiflora incarnata	0.475

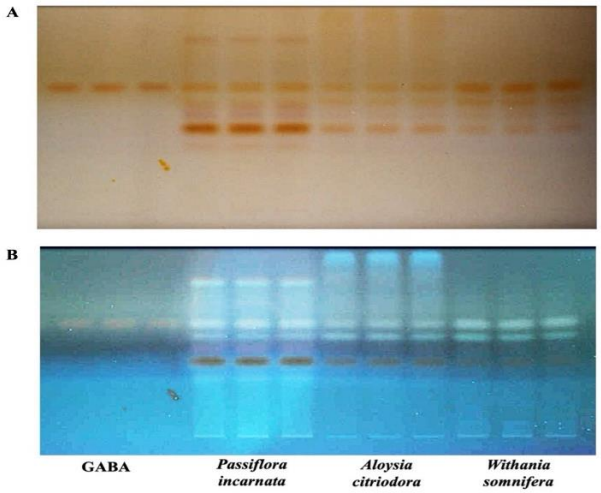

Figure 1. HPTLC fingerprinting of GABA and the plant samples. Stationary Phase: Silica gel 60 F 254 HPTLC Plate 20∗10 (cm), Mobile Phase: Butanol-acetic acid-distilled water (5:2:2), Wavelength: 550 nm. A, after spraying the HPTLC plate with ninhydrin reagent; B, after visualizing under UV.

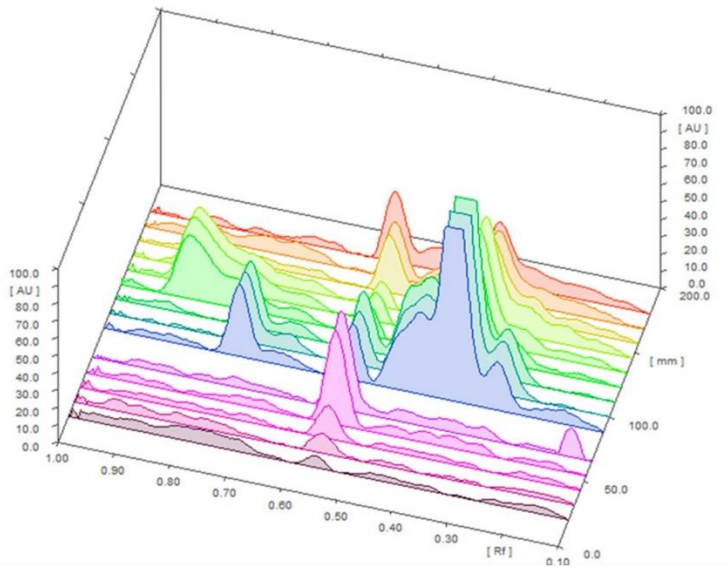

Figure 2. 3D-Chromatograms of GABA and samples. The initial five chromatograms are GABA, the blue chromatogram *Passiflora incarnata*, the green chromatogram *Aloysia citriodora*, and the red chromatogram *Withania somnifera*.

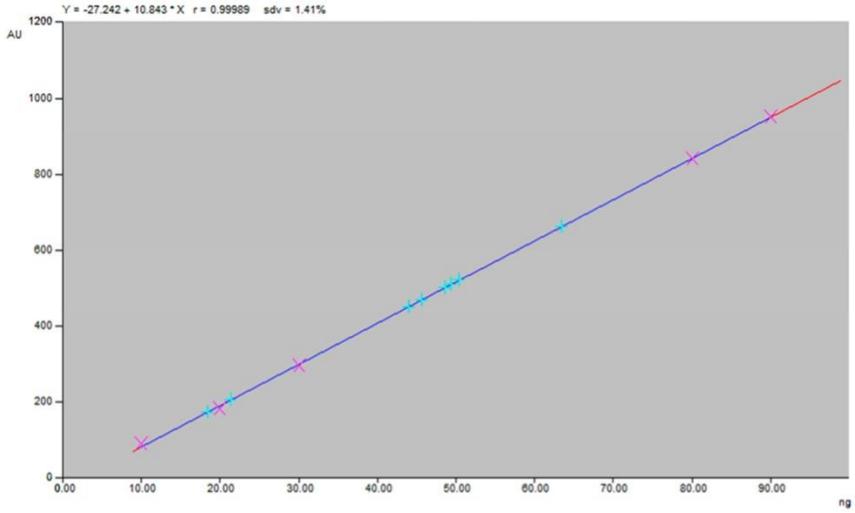

Figure 3. Calibration curve of GABA concentration based on peak areas. The blue points show the samples, and the pink ones display the standard GABA.

All five plants investigated in this study were hypnotic, and this hypnotic effect can be related to one or more substances.

Since melatonin is an indoleamine the body is almost familiar with, this compound has received much attention and entered the pharmaceutical market in different forms. Melatonin is putatively effective in reducing sleep initiation time and ameliorating the quality of sleep [13, 14, 35].

Herbal compounds containing melatonin can be suitable sleep aids because these plants may raise the level of melatonin in the blood. For example, *Tanacetum parthenium* and *Hypericum perforatum* leaves contain two ng/g of melatonin in fresh weight. *Coffea arabica*

bean contains 6800 ng/g of melatonin in dry weight [36].

Different methods detect melatonin in plants, such as ELISA, TLC, GC-MS, LC-MS, and HPLC [37]. For example, in a study conducted by Ansari and coworkers, the quantity of melatonin in extracts of *Tripleurospermum disciforme*, *Tanacetum parthenium*, and *Viola odorata* were analyzed using HPLC-UV, ELISA, and TLC [38].

Our study observed that none of the five examined plants contained melatonin, as opposed to the standard melatonin. Prior to conducting the HPLC analysis, a preliminary TLC was performed. The outcomes of the TLC analysis were in line with the results obtained from the HPLC analysis, except for the *Withania somnifera* sample. This particular sample exhibited a faint band on the HPTLC, and subsequent HPLC analysis confirmed that the substance in the band was not melatonin. It is possible that the observed band may be attributed to another indoleamine or could have resulted from an experimental error. Consequently, melatonin cannot be attributed to the hypnotic effects associated with the extract derived from these five plants.

GABA serves as the principal neurotransmitter responsible for the regulation of sleep. A significant proportion of pharmacological agents currently employed in managing sleep disorders exert their therapeutic effects through augmentation of GABAergic neuronal inhibition [39, 40]. Multiple substances derived from plants have the potential to act as sleep aids through their modulation of GABAergic signaling within the brain [41, 42]. For instance, the content of GABA in *Zingiber officinale* and *Solanum torvum* was reportedly 0.0114% and 0.0119%, respectively [43].

Three of these investigated plants, i.e., *Aloysia citriodora*, *Passiflora incarnata*, *and Withania somnifera* contained GABA, which can explain the sleep-inducing effect of these plants. Previously, the marigold plant was examined in some studies, and the amount was determined by TLC and Densitometry methods; in this case, it should be said that the extraction method is effective in determining the percentage of GABA present in the plant in one of the studies using different methods. The amount of GABA obtained varied between 2% and 3.8%; the highest amount was related to the fresh plant's ethanolic extract.

In our study, the stem and leaves of the hourglass flower were examined, and it was proved that not only the flowers of this plant but also the stem and leaves have GABA, the amount of which was equal to 0.0475%. This amount is far less than the amount of GABA in the flower of this plant. Among the three investigated plants, the root of the *Withania somnifera* plant had the highest amount of GABA, equal to 0.0495%, and the lowest amount of GABA was related to the leaves of the lemon plant, equal to 0.0214%.

4. Conclusion

In conclusion, this study described the detection and quantification of GABA in *Withania somnifera* and *Aloysia citriodora* by HPTLC for the first time. Remarkably, *Withania somnifera* showed the highest amount of GABA among the studied plants.

Acknowledgments

This research was financially supported by a grant from Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant no. 43008630) and is greatly appreciated

Conflict of interest

The authors declare to have no conflict of interest.

References

[1] De Crescenzo F, D'Alò GL, Ostinelli EG, Ciabattini M, Di Franco V, Watanabe N, et al. Comparative effects of pharmacological interventions for the acute and long-term management of insomnia disorder in adults: a systematic review and network meta-analysis. The Lancet 2022;400:170–84.

[2] Metwally A, Alalawi AD, Al Sarrar AA, Alamin OM, Saad AA, Almalki MD. Prevalence of Insomnia and Its Associated Factors in the General Population of Saudi Arabia: A Cross-Sectional Study. Cureus 2023.

[3] Hassinger AB, Bletnisky N, Dudekula R, El-Solh AA. Selecting a pharmacotherapy regimen for patients with chronic insomnia. Expert Opin Pharmacother 2020;21:1035–43.

[4] Pillai V, Roth T, Roehrs T, Moss K, Peterson EL, Drake CL. Effectiveness of Benzodiazepine Receptor Agonists in the Treatment of Insomnia: An Examination of Response and Remission Rates. Sleep 2017;40.

[5] Madari S, Golebiowski R, Mansukhani MP, Kolla BP. Pharmacological Management of Insomnia. Neurotherapeutics 2021;18:44–52.

[6] Zlott DA, Byrne M. Mechanisms by which pharmacologic agents may contribute to fatigue. PM R 2010;2:451–5.

[7] Krystal AD, Prather AA, Ashbrook LH. The assessment and management of insomnia: an update. World Psychiatry 2019;18:337–52.

[8] Talebi M, Khoramjouy M, Feizi A, Ali Z, Khan IA, Ayatollahi NA, et al. Novel multi-target therapeutic potential of the genus Inula: Advances and opportunities for neuroprotection. Pharmacological Research - Modern Chinese Medicine 2023;7:100263.

[9] Rabbani M, Vaseghi G, Sajjadi S, Amin B. Persian Herbal Medicines with Anxiolytic Properties. J Med Plants 2011;10:7–11.

[10] Jahani R, Behzad S, Saffariha M, Toufan N, Faizi M. Sedative-hypnotic , anxiolytic and possible side effects of Salvia limbata C . A . Mey . Extracts and the effects of phenological stage and altitude on the rosmarinic acid content. J Ethnopharmacol 2022;282:114630.

[11] Andalib S, Vaseghi A, Vaseghi G, Naeini AM. Sedative and hypnotic effects of Iranian traditional medicinal herbs used for treatment of insomnia. EXCLI J 2011;10:192.

[12] Feizi F, Namazi N, Rahimi R, Ayati MH. Medicinal Plants for Management of Insomnia: A Systematic Review of Animal and Human Studies. Galen Med J 2019;8:e1085.

[13] Mannino G, Pernici C, Serio G, Gentile C, Bertea CM. Melatonin and Phytomelatonin: Chemistry, Biosynthesis, Metabolism, Distribution and Bioactivity in Plants and Animals—An Overview. Int J Mol Sci 2021;22:9996.

[14] Zhang Z, Zhang Y. Melatonin in plants: what we know and what we don't. Food Qual Saf 2021;5.

[15] Chen G, Huo Y, Tan D-X, Liang Z, Zhang W, Zhang Y. Melatonin in Chinese medicinal herbs. Life Sci 2003;73:19–26.

[16] Arnao MB, Hernández- Ruiz J. Assessment of different sample processing procedures applied to the determination of melatonin in plants. Phytochem Anal 2009;20:14–8.

[17] Fan J, Xie Y, Zhang Z, Chen L. Melatonin: A Multifunctional Factor in Plants. Int J Mol Sci 2018;19:1528.

[18] Ramos-Ruiz R, Martinez F, Knauf-Beiter G. The effects of GABA in plants. Cogent Food Agric 2019;5:1670553.

[19] Li L, Dou N, Zhang H, Wu C. The versatile GABA in plants. Plant Signal Behav 2021;16:1862565.

[20] Bouché N, Fromm H. GABA in plants: just a metabolite? Trends Plant Sci 2004;9:110–5.

[21] Bown AW, Shelp BJ. Plant GABA: Not Just a Metabolite. Trends Plant Sci 2016;21:811–3.

[22] Pencheva D, Teneva D, Denev P. Validation of HPLC Method for Analysis of Gamma-Aminobutyric and Glutamic Acids in Plant Foods and Medicinal Plants. Molecules 2022;28:84.

[23] Bahramsoltani R, Rostamiasrabadi P, Shahpiri Z, Marques AM, Rahimi R, Farzaei MH. Aloysia citrodora Paláu (Lemon verbena): A review of phytochemistry and pharmacology. J Ethnopharmacol 2018;222:34–51.

[24] Afrasiabian F, Mirabzadeh Ardakani M, Rahmani K, Azadi NA, Alemohammad ZB, Bidaki R, et al. *Aloysia citriodora* Palau (lemon verbena) for insomnia patients: A randomized, double- blind, placebo- controlled clinical trial of efficacy and safety. Phytother Res 2019;33:350–9.

[25] Bahramsoltani R, Rostamiasrabadi P, Shahpiri Z, Marques AM, Rahimi R, Farzaei MH. Aloysia citrodora Paláu (Lemon verbena): A review of phytochemistry and pharmacology. J Ethnopharmacol 2018;222:34–51.

[26] Chiavaroli A, Di Simone S, Acquaviva A, Nilofar, Libero M, Brunetti L, et al. Neuromodulatory and Protective Effects Induced by the Association of Herbal Extracts from Valeriana officinalis, Ziziphus jujuba, and Humulus lupulus with Melatonin: An Innovative Formulation for Counteracting Sleep Disorders. Processes 2022;10:1609.

[27] Abdellah SA, Berlin A, Blondeau C, Guinobert I, Guilbot A, Beck M, et al. A combination of Eschscholtzia californica Cham. and *Valeriana officinalis* L. extracts for adjustment insomnia: A prospective observational study. J Tradit Complement Med 2020;10:116–23.

[28] Pereira Leal AEB, de Lavor ÉM, de Menezes Barbosa J, de Moura Fontes Araújo MT, dos Santos Cerqueira Alves C, de Oliveira Júnior RG, et al. Pharmacological Activities of the Genus Passiflora (Passifloraceae): A Patent Review. Curr Top Med Chem 2022;22:2315–28.

[29] Mikulska P, Malinowska M, Ignacyk M, Szustowski P, Nowak J, Pesta K, et al. Ashwagandha (*Withania somnifera*)—Current Research on the Health-Promoting Activities: A Narrative Review. Pharmaceutics 2023;15:1057.

[30] Park CW, Hong K-B, Suh HJ, Ahn Y. Sleep-promoting activity of amylase-treated Ashwagandha (*Withania somnifera* L. Dunal) root extract via GABA receptors. J Food Drug Anal 2023;31:27–288.

[31] Luo J, Jiang W. A critical review on clinical evidence of the efficacy of lavender in sleep disorders. Phytother Res 2022;36:2342–51.

[32] Huang X, Mazza G. Application of LC and LC-MS to the Analysis of Melatonin and Serotonin in Edible Plants. Crit Rev Food Sci Nutr 2011;51:269–84.

[33] Cao J, Murch SJ, O'Brien R, Saxena PK. Rapid method for accurate analysis of melatonin, serotonin and auxin in plant samples using liquid chromatography–tandem mass spectrometry. J Chromatogr A 2006;1134:333–7.

[34] Roshanravan B, Yousefizadeh S, Apaydin Yildirim B, Farkhondeh T, Amirabadizadeh A, Ashrafizadeh M, et al. The effects of Berberis vulgaris L. and Berberis aristata L. in metabolic syndrome patients: a systematic and meta-analysis study. Arch Physiol Biochem 2023;129:393–404.

[35] Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol 2018;175:3190–9.

[36] Salehi B, Sharopov F, Fokou P, Kobylinska A, Jonge L, Tadio K, et al. Melatonin in Medicinal and Food Plants: Occurrence, Bioavailability, and Health Potential for Humans. Cells 2019;8:681.

[37] Meng X, Li Y, Li S, Zhou Y, Gan R-Y, Xu D-P, et al. Dietary Sources and Bioactivities of Melatonin. Nutrients 2017;9:367.

[38] Ansari M, Kh R, Yasa N, Vardasbi S, Naimi SM, Nowrouzi A. Measurement of melatonin in alcoholic and hot water extracts of Tanacetum parthenium, Tripleurospermum disciforme and Viola odorata. DARU Journal of Pharmaceutical Sciences 2010;18:173.

[39] Plante DT, Jensen JE, Winkelman JW. The Role of GABA in Primary Insomnia. Sleep 2012;35:741–2.

[40] Hepsomali P, Groeger JA, Nishihira J, Scholey A. Effects of Oral Gamma-Aminobutyric Acid (GABA) Administration on Stress and Sleep in Humans: A Systematic Review. Front Neurosci 2020;14.

[41] Bruni O, Ferini-Strambi L, Giacomoni E, Pellegrino P. Herbal Remedies and Their Possible Effect on the GABAergic System and Sleep. Nutrients 2021;13:530.

[42] Pei L, Zhao Y, Shi X, Chen R, Yan J, Li X, et al. The Role of γ-Aminobutyric Acid (GABA) in the Occurrence of Adventitious Roots and Somatic Embryo s in Woody Plants. Plants 2022;11:3512.

[43] Rema Shree AB, Anju P, Moothedath I. Gamma amino butyric acid accumulation in medicinal plants without stress. Anc Sci Life 2014;34:68.