Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38(2)

Measuring the Amount of Melatonin in Twelve Medicinal and Edible Plants

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Received: 13 June 2023 Accepted: 16 August 2023 Published: 20 April 2025
doi.org/10.30526/38.2.3600

Abstract

Melatonin (Mel) is a conserved chemical that has been found in far-removed evolutionary organisms. Additionally, it has been proposed that Mel is a stand-alone plant growth regulator whose action is presumably similar to that of IAA and may moderate the effects of other plant growth regulators. Twelve medicinal herbs were divided into parts, and their melatonin concentrations were measured. These plants *Ziziphus spina-christi*, , *Eucalyptus comaldulensis, Melissa officinalis, Alhagi maurorum, Lavandula angustifolia, Vitex agnus-sactus, Piper nigrum, Hordeum vulgare, Matricaria chamomilla, Borago officinalis, Solanum lycopersicum* and *Citrus aurantium* . Methanol was used to extract melatonin from dried plant parts at room temperature for 6 hours. Then, cyclo (18) carbon was used to refine melatonin. Then, melatonin was analyzed with an reversed-phase chromatography (RP-C18)-approved high-performance liquid chromatography system "HPLC" technique with fluorescence detection and Thin layer chromatography "TLC". Melatonin contents in extracts of *Z.spina-christi, E. comaldulensis, M. officinalis, A. maurorum, L. angustifolia,V. agnus-sactus, P. nigrum, H. vulgare, M. chamomilla, B. officinalis, S. lycopersicum and C. aurantium* were 420.494, 418.831, 409.750, 408.248, 385.145,239.967, 232.299, 220.569,215.804, 201.862, 108.517 and 106.744 ng/g of plant dry weight, respectively. The extract of *Z. spina-christi* contained the most melatonin (420.494 ng/g of plant dry weight). Not only do Mel concentrations vary between species, but also within variants of the same species. Different environmental influences on plant growth and development during successive stages of seed morphological and physiological development may cause variations in MEL contents.

Keywords: Melatonin, HPLC, TLC

1. Introduction

The compound known as indole amine (melatonin) with the chemical formula N-acetyl-5-methoxytryptamine was first discovered and extracted from the pineal gland of bovine animals in 1958. This groundbreaking discovery was made by a team of scholars led by Lerner, who worked at Yale University, located in the city of New Haven, Connecticut. (1). Organisms evolutionarily distinct from humans, such as bacteria, higher plants, invertebrates, and vertebrates, have observed melatonin, a conserved compound (2, 3). Numerous higher plant

organs, including roots, stems, leaves, flowers, fruits, and seeds, contain varying amounts of this indole amine (4, 5). From a physio-chemical perspective, the pure form of Mel exhibits characteristics of an off-white powder, with a molecular weight of 232.28 g/mol and a density of 1.175 g/cm3. Mel's melting and boiling points range from 116.5°C to 118°C (6). The chemical formula C13H16N2O2 recognizes the compound known as melatonin. It has a chemical structure called indole that has been modified with both a 3-amide group and a 5-alkoxygroup. Furthermore, because it originates from a tryptophan molecule, we classify it as an indolamine compound (7). The chemical structure in question bestows exceptional stability through high resonance mesmerism. The 3-amide group and the 5-alkoxygroup are also very important in making this molecule amphiphilic. This property makes it possible for melatonin to pass through biological membranes and reach all cellular and subcellular spaces (8). This makes it easy for it to spread and protects many cell spaces from oxidative stress (9).

Research has demonstrated that the organelles of utmost melatonin concentration in plants are mitochondria and chloroplasts (10). Taking this observation along with the fact that serotonin N-acetyltransferase (SNAT), an important enzyme in the biosynthesis of melatonin, has been found in chloroplasts (11, 12) and mitochondria (13), it suggests that these are the main places where this indolamine is made. Numerous plant species have identified the genes encoding all the enzymes that facilitate the entire melatonin biosynthetic pathway, except for one potential gene that encodes for a tryptophan hydroxylase (TPH), which catalyzes the conversion of tryptophan to 5-hydroxytryptophan (14). Vertebrates have already established the enzyme in question, but plants have only recently suggested its existence. Plants initiate the biosynthesis of melatonin with the amino acid tryptophan, synthesized de novo through the shikimate pathway. The shikimate pathway comprises seven distinct steps, enabling the biosynthesis of all aromatic amino acids in plants, encompassing tryptophan (15). During the process of plant senescence or when exposed to abiotic stressors, it has been observed that plants have a tendency to amass substantial quantities of melatonin intermediates, including tryptophan, tryptamine, and serotonin, as reported in reference (16).

Finally, it is plausible that alternative biosynthetic pathways for melatonin may be present (17). The primary objective of the present investigation was to ascertain economical sources of melatonin, which was achieved by assessing diverse botanical residues for the existence of Mel.

2. Materials and Methods

2.1. Plant materials

Mature part of Z. spina-*christi, Eucalyptus, M. officinalis, Alhagi, L. angustifolia, V. agnus-sactus, P. nigrum, H. vulgare, M. chamomilla, B. officinalis, S. lycopersicum, and C. aurantium* during November 2021. All plant specimens were collocated from Iraq-waist (**Table 1**). The specimens were pulverized into fine particulates employing a blender subsequent to desiccation for a six-hour interval within a hot air oven maintained at a temperature of 60 °C. Roughly 10% of the original weight of the plant's segment represented its dehydrated mass. Sigma Corporation, located in St. Louis, MO, USA, offered the melatonin benchmark and additional chemicals.

2.2. Sample preparation and extraction

The previously stated process was adjusted for extraction and analysis (18). We filled 250 mL rotating containers with 80 g of herbal powders and 250 mL of methanol, then left them at room

temperature for six hours. Following centrifugation, we separated the resulting supernatants and stored them at 4°C for further analysis using techniques like TLC and HPLC.

Table 1. Wastes of plants used in this study.

Scientific name	Plant Parts
Ziziphus spina-christi	Leaves
Eucalyptus comaldulensis	Leaves
Melissa officinalis	Leaves
Alhagi maurorum	leaves
Lavandula angustifolia	Leaves
Vitex agnus-sactus	Flowers
Piper nigrum	Seeds
Hordeum vulgare	Seeds
Matricaria chamomilla	Flowers
Borago officinalis	Leaves
Solanum lycopersicum	Fruits
Citrus aurantium	Peels

2.3. Determination of MEL concentration

Upon completion of the extraction method, the identification of melatonin within each extract was achieved through Thin Layer Chromatography (TLC). Furthermore, the concentration of this hormone was assessed and quantified using the HPLC-fluorescence system, as elaborated below.

2.4. HPLC Analysis of Melatonin Using the HPLC-fluorescence system (HPLC-FL)

The cyclo (18) carbon (C18) column Sykam produced in Germany (2013) injector and 20-l sample loop make up the HPLC apparatus. In order to calculate the melatonin retention period, a wavelength of 280 nm was used. The mobile phase had a flow rate of (1) ml/min and was made up of 20% acetonitrile and (0.1) M potassium phosphate buffer "pH 4.5" (19).

2.5. Thin layer chromatography (TLC) analysis

Early studies (HPLC) produced solutions of standard melatonin found in the extracts under study and placed them onto TLC sheets. 20 x 20 cm DC sheets of aluminum and silica gel 60 F254 F254 Kieselgel 60 TLC 60 F254 CCM Silica gel Silica gel 60 F254. At this stage of the research, we were optimizing the conditions for the chromatographic separation of the chemicals under study. We separated the compounds using a mobile phase consisting of butan-1-ol, glacial acetic acid, water (12: 3: 5, v/v/v), and melatonin (Rf 0.81). On chromatograms, which were later dried at room temperature, spots were located using UV. At 280 nm, densitometric analysis was carried out. (**Figures 1, 2,** and **3**)(20, 21).

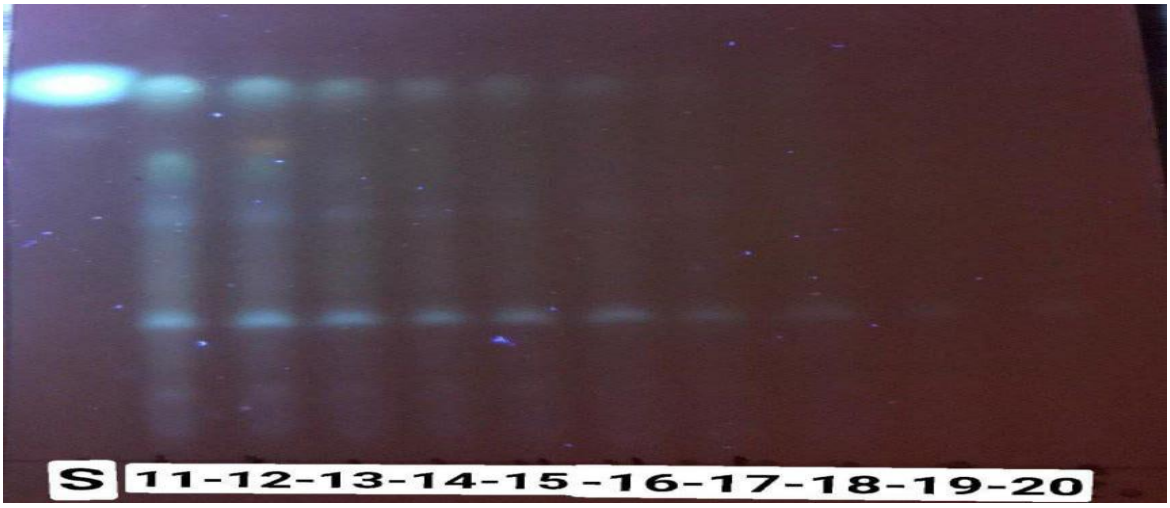

Figure 1. TLC of Ziziphus spina-christi, S=stander, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 tubes contain melatonin after purification on C18 column.

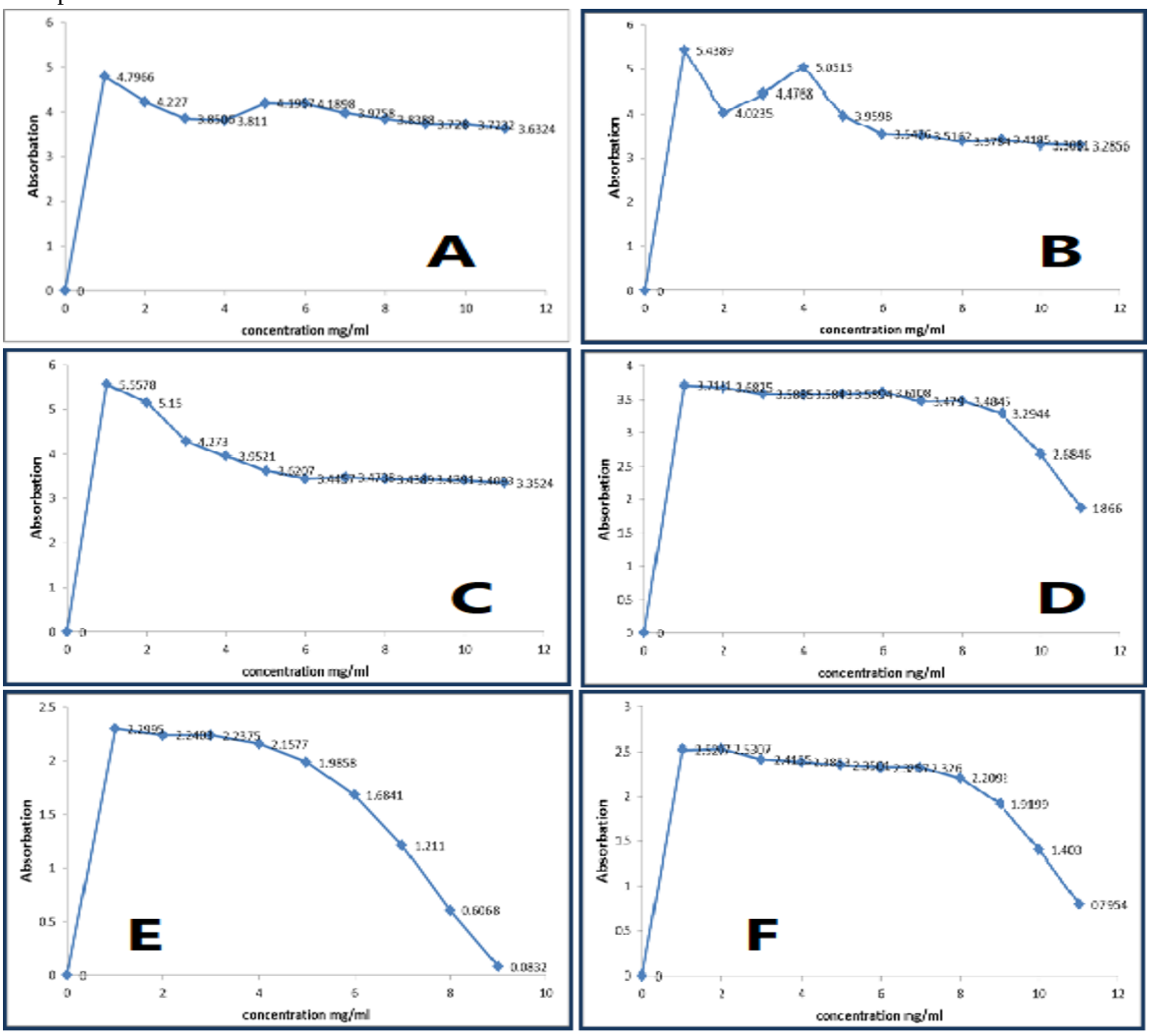

Figure 2. Curve after purification of (A- *Ziziphus spina-christi*, B-*Eucalyptus comaldulensis*, *C-Matricaria chamomilla*, D- *Hordeum vulgare*, E- *Solanum lycopersicum*, F- *Alhagi maurorum*) on C18 column.

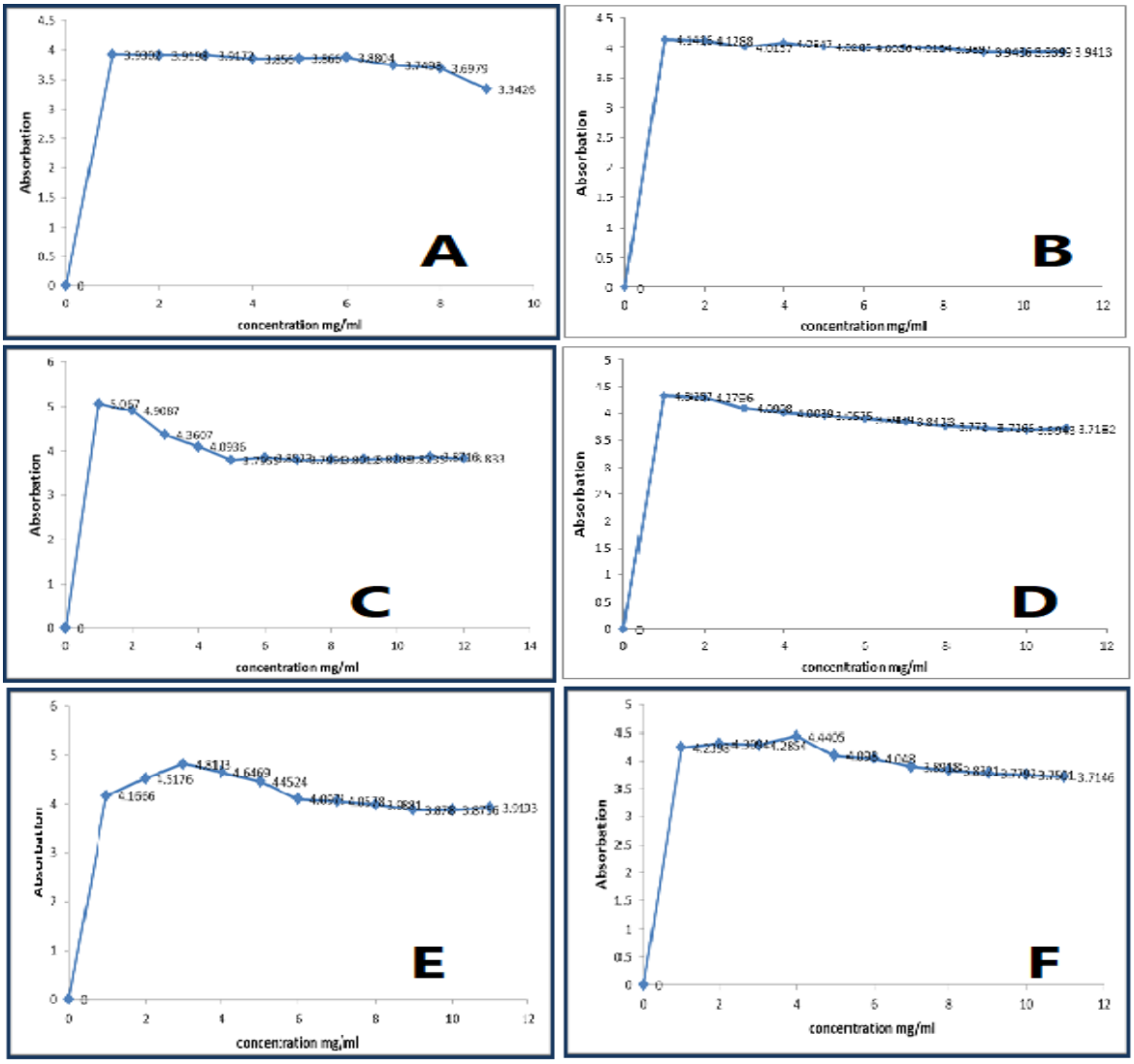

Figure 3. Curve after purification of (A- *Piper nigrum*, B-*Vitex agnus-sactus*, C- *Melissa officinalis*, D- *Lavandula angustifolia*, E -*Citrus aurantium*, F -*Borago officinalis*) on C18 column.

2.6. Standard preparation

A solution of Mel stock, at a concentration of 1 mg/mL, was formulated by dissolving 100 mg of Mel standard in 100 mL of methanol and normal saline, which was then stored at a temperature of -20 °C. To obtain the working standard solutions, which had concentrations of 0.25, 0.35, 0.45, 0.55, 0.01, 0.3, 0.4, 0.5, 0.6, and 0.7 µg/mL, the stock solution was diluted with methanol and normal saline. These working solutions were freshly prepared each day.

In this study, standard solutions were added to the HPLC-Fl system, and Mel concentrations were plotted against the area under their peaks to make a calibration curve. The obtained equation Y = 1.6502x, R2 = 0.769 was utilized to estimate the concentration of Mel in each sample. Notably, Mel is known to be vulnerable to oxidation by free radicals; therefore, it is imperative to protect the samples from light during all extraction procedures to avoid photo-oxidation (22) (**Figure 4**).

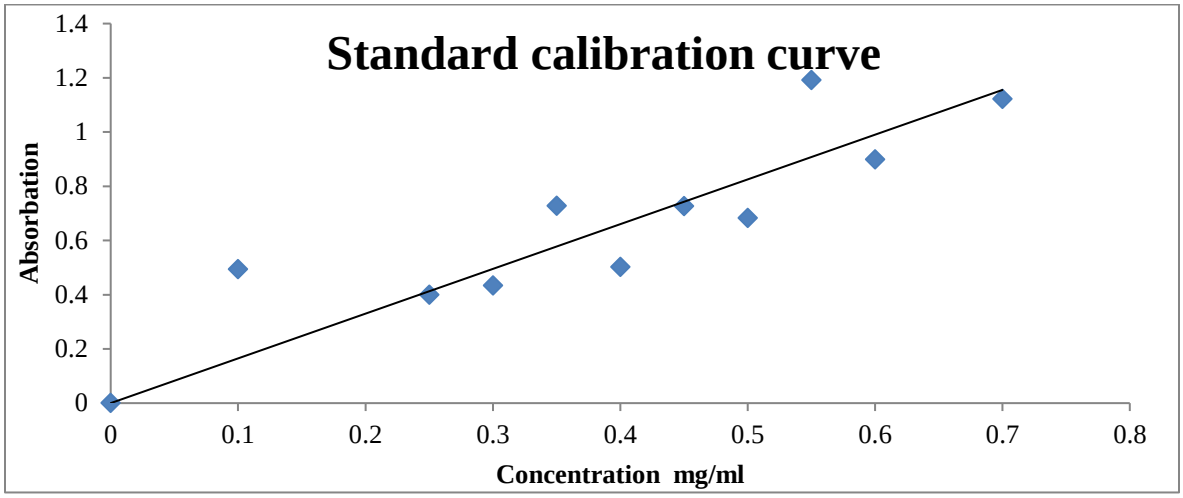

Figure 4. Melatonin standard calibration curve at 280 nm.

3. Results and Discussion

3.1. Determination of the retention time of melatonin using the (HPLC-fluorescence) system

In this study, the process of extracting Mel from a variety of twelve medicinal and edible plants was conducted. The presence of Mel in these extracts was determined via employment of an HPLC-fluorescence system, with the retention time of this compound being measured through the use of standard Mel. An illustration of these findings can be seen in **Figure 5**. Evidently, from this chromatogram, it is apparent that the retention time of Mel under the experimental conditions employed is equivalent to 4.433 minutes.

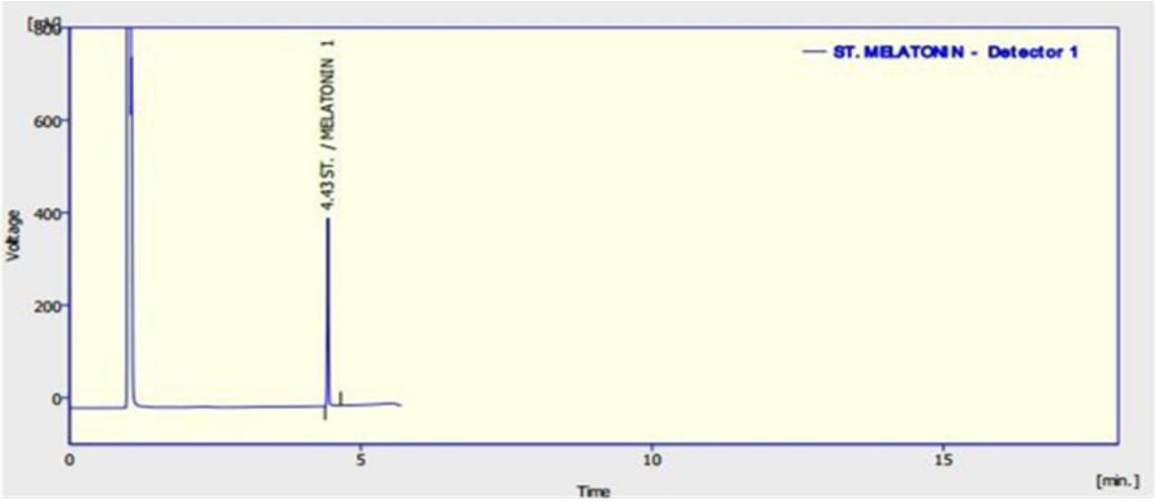

Figure 5. HPLC melatonin standard Fluorescent detector, nm= 280, mobile phase 20% acetonitrile in potassium phosphate buffer at pH 4.5.

3.2. Determination of melatonin concentration in the twelve medicinal and edible plants

Determination of melatonin concentration in the twelve medicinal and edible plants. The findings presented in **Table 2** demonstrate that *Ziziphus spina-christi* leaves yield the highest Mel extraction among the studied plants. Conversely, the remaining plants exhibit lower Mel content. Chromatogram analysis of the extract obtained from *Ziziphus spina-christi* is depicted in

Figures 6 and **7**. Melatonin concentrations vary not only across different species but also among distinct varieties of the same species, as previously reported (23).

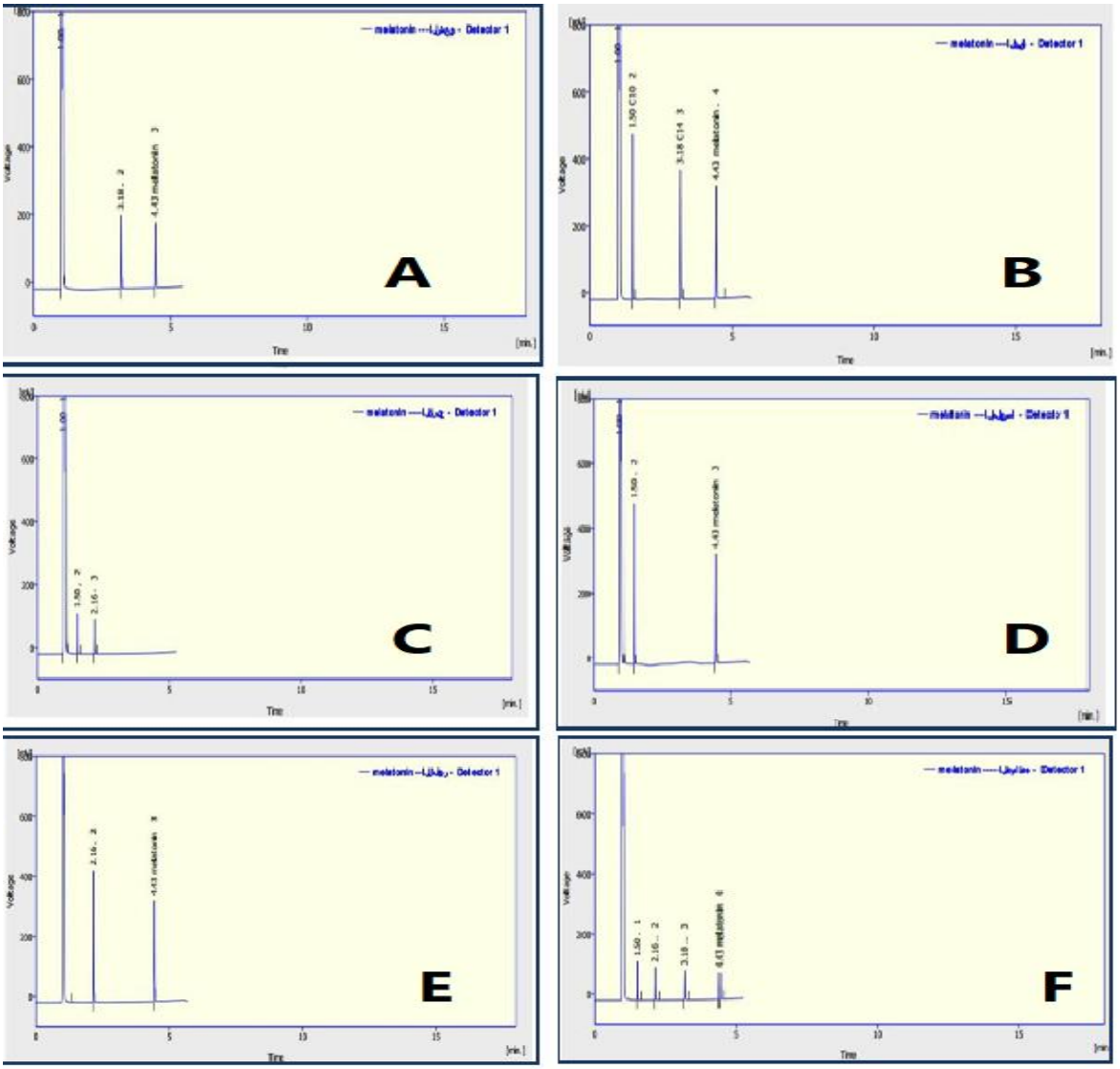

Figure 4. A. Negative control, B. Ps m isolate. C. Ps 81 isolate.

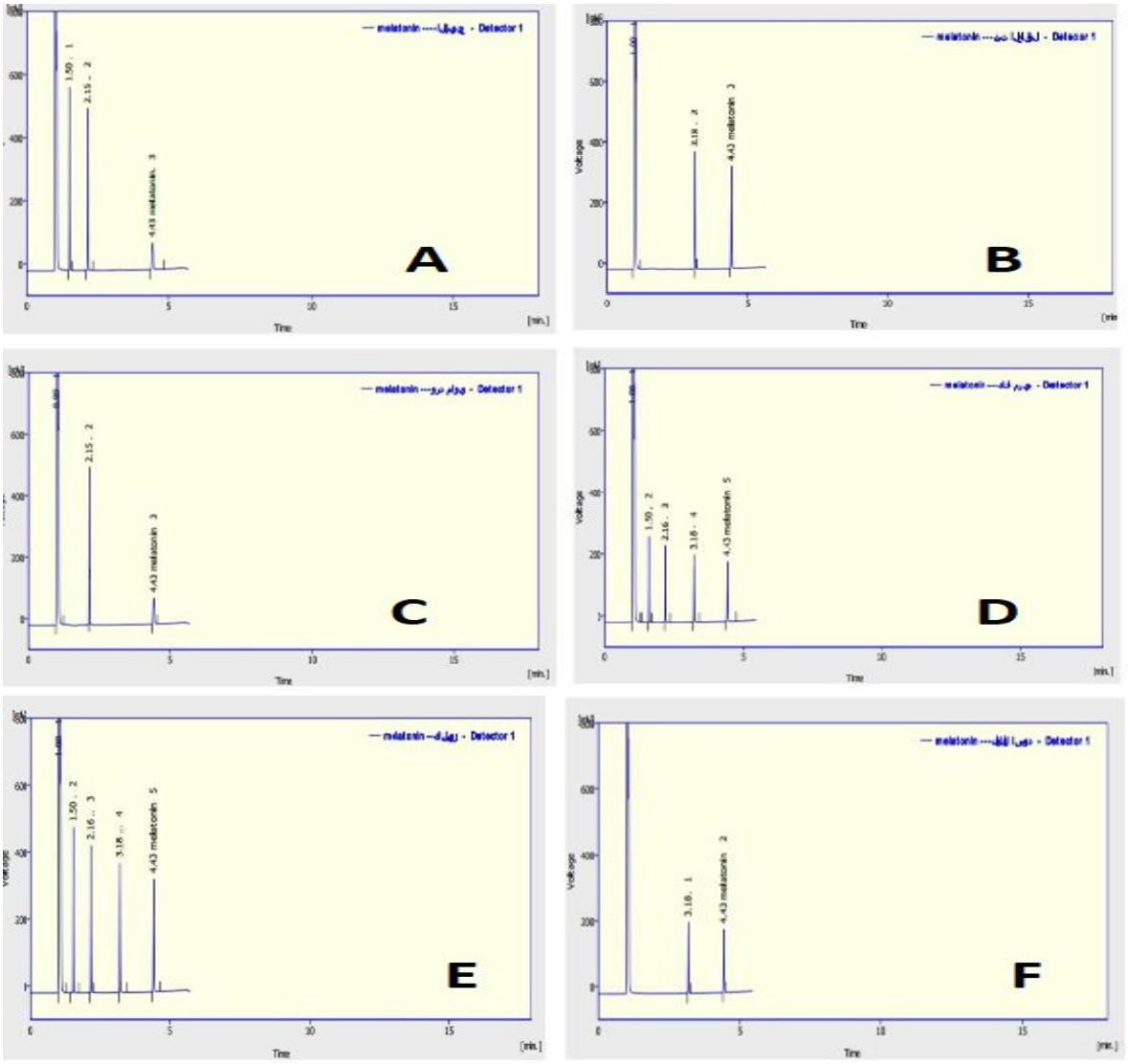

Figure 7. HPLC. Fluorescent of (A-*Matricaria chamomilla*, B-*Alhagi maurorum*, C-*Borago officinalis*, D- *Vitex agnus-sactus*, E-*Eucalyptus comaldulensis*, F- *Piper nigrum*) detector, nm= 280, mobile phase 20% acetonitrile in potassium phosphate buffer at pH 4.5.

Table 2. Melatonin contents and area in medicinal plant determined by HPLC.

Plant	Plant Parts	Amount (ng/g)	Response (area)
Ziziphus spina-christi	Leaves	420.494	553.000
Eucalyptus comaldulensis	Leaves	418.831	550.812
Melissa officinalis	Leaves	409.750	538.871
Alhagi maurorum	leaves	408.248	536.895
Lavandula angustifolia	Leaves	385.145	506.511
Vitex agnus-sactus	Flowers	239.967	315.586
Piper nigrum	Seeds	232.299	305.501
Hordeum vulgare	Seeds	220.569	290.075
Matricaria chamomilla	Flowers	215.804	283.808
Borago officinalis	Leaves	201.862	265.473
Solanum lycopersicum	Fruits	108.517	138.634
Citrus aurantium	Peels	-	-

The presence of Mel in the parts of these plants has been confirmed through its co-elution with standard Mel, as shown in **Figures 6** and **7**. Significant variations in the concentrations of Mel

were observed across different samples. The reliability of the data obtained can be attributed to the efficacy of the applied technique, which is dependent on the chemical properties of the extraction solvent and homogenate buffer, such as their polarity and radical scavenging capabilities (22, 24). The selection of suitable Mel physiochemical properties and location within the cell is of utmost importance. The efficacy of the utilized method is directly related to the increase in MEL solubility and its protection from the harmful effects of free radicals on plant species and the specific part of the plants being studied (as presented in **Table 2, Figures 6** and **7**). It was discovered that *Z.*spina-*christi* leaves exhibit a high concentration of Mel, while the concentrations in other plant extracts were significantly lower with a substantial disparity.

Depending on the results obtained from the employed extraction methodology, it is plausible that Mel may be detected in *Solanum lycopersicum* fruit and *Citrus aurantium* peels at a significantly low concentration. The leaves of *Z.spina-christi* and *Eucalyptus comaldulensis* exhibited the highest concentrations of Mel, as indicated in **Table 2**, and **Figures 6** and **7**, registering at 420.494 and 418.831 ng Mel/gm, respectively. Conversely, the peels of *Citrus aurantium* showed minimal or insignificant levels of Mel. This contradicts the findings of research by (22, 25), and this observation may be attributed to multiple factors, including the specific plant part utilized, geographical region of origin, storage conditions, plant species, stage of ripeness, the solvent employed for extraction, and variations in the sensitivity of the quantification assays utilized in previous analyses (26). Finally, based on the findings of the present study, it is possible to consider the utilization of *Z. spina-christi* leaves as a cost-effective source for the production of this indole compound, which showcases numerous applications across various domains. Additionally, this research offers a means for recycling this part of plants. Further investigations are currently underway in our laboratory to examine the waste of alternative plant species for the detection of this compound (27).

5. Conclusion

Melatonin was identified in all the samples and the commercial preparation. Additionally, Mel may also aid in the formation of a differentiated state in fruit tissue or the maintenance of germ tissue dormancy. In the future, it would be prudent to explore and assess the melatonin concentration in various foods to identify fresh natural sources of the same. Additionally, there is a need to conduct more in-depth research to comprehend the mechanisms of action. Furthermore, conducting a greater number of clinical trials would aid in elucidating the impact of melatonin on humans. Meanwhile, it would be highly advantageous to develop certain foods that possess exceptionally high melatonin content into functional foods, which could aid in the prevention and treatment of diverse ailments.

Acknowledgment

Many thanks to the Department of Biology at the College of Science, University of Baghdad, for their invaluable assistance in facilitating the practical sections of this article.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No funding.

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