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Melatonin Extraction and Determination by UHPLC-FD from Different Plants

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ABSTRACT: Many plants have become a piece of the traditional food as human diet and evaluated in folk medicine to treat diseases. They are very rich in terms of nutraceuticals. Melatonin functions as a free radical scavenger and controls the regulation of the sleep–wake cycle in mammals. In this study, we analyzed melatonin content in the herba parts of *Stachys lavandulifolia*, *Tribulus terrestris*, and *Helichrysum arenarium*, and unripe fruits of *Prunus armeniaca* L., *Prunus cerasifera*, and *Prunus amygdalus* L. A solvent mixture of methanol:ultrapure water:HCl (70:29.9:01 v/v/v) was used for the extraction of melatonin from plant samples. The determination of melatonin in the extracts was carried out by high pressure liquid chromatography with fluorescence detector (UHPLC-FD). Melatonin concentration was 0.0231 mg/kg for herba of *Helichrysum arenarium* and 0.0018 mg/kg for *Prunus armeniaca* L. Melatonin was not detected in other plant samples.

Keywords: *Helianthus annuus, Prunus armeniaca L., Melatonin*

1 INTRODUCTION

Melatonin, a hormone primarily known to its role in regulating the sleep-wake cycle in humans, has emerged as a fascinating compound found not only in animals but also abundantly in various plants and fruits. This naturally occurring substance has garnered considerable interest due to its potential health benefits beyond sleep regulation. Understanding the presence and significance of melatonin in medicinal plants and selected fruits provides insights into its broader physiological roles and therapeutic potential.

In recent years, research has increasingly focused on identifying melatonin in botanical sources, revealing a diverse array of plant species that produce this hormone. This discovery underlines that melatonin is not only an endogenous mammalian hormone and its importance in plant physiology and ecology.

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring indoleamine hormone produced within the body. In mammals, melatonin serves as a biological regulator

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affecting various functions, including mood, sleep, hormone regulation, immune responses, circadian rhythms, and sexual behavior [1]. Identified in plants in 1995, melatonin also plays a role in processes such as photoperiodicity, flowering, and growth [2]. Additionally, it acts as a powerful antioxidant by neutralizing free radicals and enhancing antioxidant enzyme activity [3]. Research into melatonin's therapeutic benefits has revealed its wide range of bioactivities, including anticancer effects, anti-inflammatory properties, cardiovascular protection, immune system support, antidiabetic effects, neuroprotection, anti-aging benefits, and overall antioxidant activity [4–6]. Studies suggest that consuming melatonin-rich foods can elevate serum melatonin levels and improve antioxidant capacity in humans [7].

The use of plants dates back to ancient times, long before the advent of agriculture [8], and provides numerous advantages and opportunities for societies [9]. Many studies have shown the presence of melatonin in different plant species and tissues such as *Lupinus albus* L., *Hordeum vulgare* L. [10], *Prunus cerasus* [11], *Nicotiana tabacum* [12], *Hypericum perforatum* [13], *Helianthus annuus*, *Brassica nigra*, *Apium graveolens* [14], *Lycium barbarum*, and *Morus rubra* [15]. But the melatonin content of many plants is still unknown. In this study, the melatonin content of the herbal parts of three herbaceous plants (*Stachys lavandulifolia*, *Tribulus terrestris*, and *Helichrysum arenarium*) and unripe fruits of three stone fruit species (*Prunus armeniaca* L., *Prunus cerasifera*, and *Prunus amygdalus* L.) were investigated.

2. MATERIAL AND METHOD

2.1 Chemicals

The solvents methanol, ethanol, acetonitrile, and formic acid were supplied from Merck (Darmstadt, Germany) and were of HPLC analytical grade. Sodium acetate (≥99% purity) and melatonin (pure reagent) from Merck (Darmstadt, Germany) were purchased. Ultrapure water was prepared by a Millipore Direct-Q 3 UV-R water purification system (Molsheim, France).

2.2 Plant Material

In this study, *Stachys lavandulifolia*, *Tribulus terrestris*, and *Helichrysum arenarium* consumed as tea and *Prunus armeniaca* L., *Prunus cerasifera*, and *Prunus amygdalus* L. from stone fruits were used as plant materials.

Stachys lavandulifolia is known for its strong anti-oxidant effect, mild calming, and appetizing tea. This herb is also used to reduce anxiety and cure gastrointestinal

disorders [16-18]. *Tribulus terrestris* has been used in folk medicine throughout history to treat disorders such as impotence, rheumatism, edema, hypertension, and kidney stones [19]. *Helichrysum arenarium* is an herb known for its cholagogue, choleretic, hepatoprotective, and detoxifying properties, which have an important place in the traditional medical practice of Europe. The plant supports liver health by helping to remove harmful substances from the body and regulates the digestive system [20]. *Prunus armeniaca* is used for different purposes in various cultural and medical traditions. In particular, apricot seeds and oils are used to treat gynecological diseases, rheumatic pains, headaches, and skin hyperpigmentation. It is also used as a decoction for diseases like asthma, cough with phlegm, and fever. In China and other Asian countries, it is used in traditional medicines for viral infections and respiratory problems [21]. *Prunus cerasifera* fruit is rich in vitamins A, B1, B2, and C and sugar and can be used in diets. It is good for skin, hair, and eye health, besides, fresh plums are a good tonic for the kidney and stomach and are good for arthritis and rheumatism. In modern medicine, dried plums are powdered and used for coughs, colds, and sore throats. The oil obtained from the seeds of some plum varieties is very good for the beauty of the skin [22]. *Prunus amygdalus* is considered nutritious as it is a rich source of fat and protein. As part of its nutritional importance, it has also been reported to have beneficial effects on cholesterol levels and lipoprotein profiles in human blood, particularly lowering low-density lipoprotein (LDL) cholesterol [23-25].

Fruit samples from a farmer's garden in Malatya and other plant samples from a spice shop in Bingöl were obtained.

2.3 Extraction Procedure

The homogenized plant samples were extracted in the solvent mixture of methanol: ultrapure water: HCI (70:29.9:0.1 v/v/v). The samples (10.00 g) was extracted with methanol:ultrapure water:HCI (100 mL) on a ultrasonic bath for 1 hours and 37°C. The obtained extracts were concentrated with the help of rotavapor under low pressure and 40 °C. The resulting dry extract was dissolved in 5 mL of 90% methanol and then was filtered through a 0.45 µm PVDF (polyvinylidene difluoride) filter. This supernatant was used in analyses of melatonin.

2.4 UHPLC-FD analysis of melatonin

Chromatographic studies were quantified by UHPLC (Shimadzu Technologies, Kyoto, Japan), equipped with a RF-20 A

model fluorescence detector (FD). Analytical separations were performed using a Welch Welchrom C18 5 mm reversed-phase column (250 mm×4.6 mm). Isocratic elution was performed with 1 mL/min flow rate at 25°C, and the injection volume was 20 µL. The solvent mixture of methanol: water: formic acid (55:44.9:0.1 v/v/v) was used as the mobile phase, and the fluorescence detector were measured at the excitation/ emission wavelengths 275/345 nm [26]. The analytical parameters for melatonin are given in Table 1.

Table 1. Analytical parameters for melatonin analysis

Compound	Retention time	Linear equation	Linear range (mg/L)	R^2
Melatonin	5.134	y=799.62x+68.507	0.01-0.5	0.9996

3. RESULT

In the present study, it was investigated the melatonin content of the herba parts of *Stachys lavandulifolia*, *Tribulus terrestris*, and *Helichrysum arenarium*, and the unripe fruits of *Prunus armeniaca* L., *Prunus cerasifera*, and *Prunus amygdalus* L. To quantify melatonin content, the melatonin standart curve was established using the UHPLC-FD system within the range of 0.01-0.5 mg/L (Figure 1), and the results expressed as mg/kg.

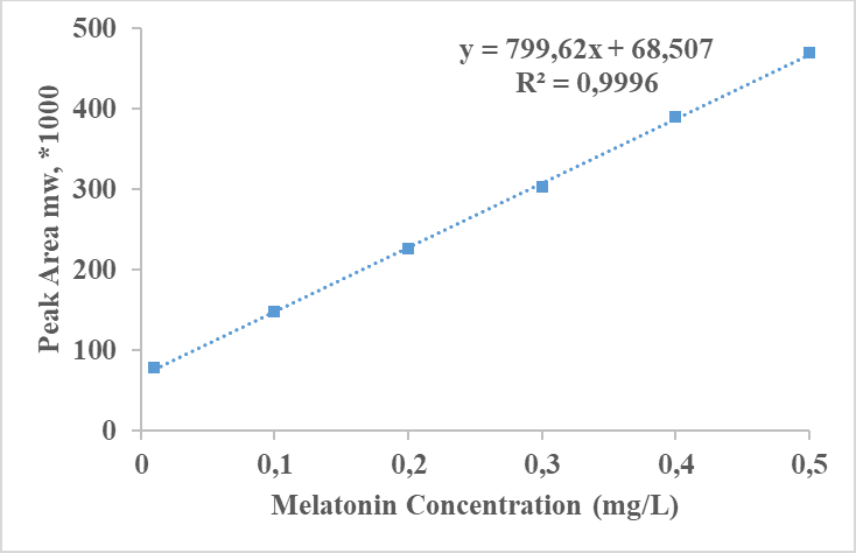

Figure 1. Calibration curve for the melatonin.

Melatonin concentration for plant samples was calculated based on the UHPLC peak areas. Melatonin results were presented in Table 2. Between herbaceous plants, melatonin was detected only in *Helichrysum arenarium*, while melatonin wasn't detected in *Stachys lavandulifolia* and *Tribulus terrestris*. Similarly, melatonin was detected only in *Prunus armeniaca* L. among stone fruit species, whereas it was not detected in *Prunus cerasifera* and *Prunus amygdalus* L.

Table 2. Melatonin content of plant samples (mg/kg)

Plant Samples (Herba)	Melatonin Content	Plant Samples (Unripe Fruit)	Melatonin Content
Stachys lavandulifolia	ND	*Prunus armeniaca* L.	0.0018±0.00
Tribulus terrestris	ND	*Prunus cerasifera*	ND
Helichrysum arenarium	0.0231±0.002	*Prunus amygdalus* L.	ND

ND: Not Dedection

4 DISCUSSION

In recent years, there has been increased interest in determining the roles of plant-derived compounds in improving human health. Plants are splendid sources of bioactive compounds and nutrients. Epidemiological studies have emphasized the importance of consuming edible plants on the prevention of many human diseases [27]. In mammals, melatonin acts as a biological regulator affecting numerous processes, including immune responses, mood, circadian rhythms, hormone regulation, sexual behavior, and sleep. This natural hormone offers several health benefits, such as enhancing antioxidant enzyme activity and serving as a strong antioxidant that neutralizes free radicals. In plants, melatonin plays roles in flowering, photoperiodicity, growth, development, and boosting resilience to environmental stressors [28-30]. In the present study, the melatonin content was examined in the herba parts of *Helichrysum arenarium*, *Stachys lavandulifolia*, *Tribulus terrestris*, and unripe fruits of *Prunus armeniaca* L., *Prunus cerasifera*, and *Prunus amygdalus* L. Different amounts of melatonin were detected in *Helichrysum arenarium* and *Prunus armeniaca* L. plant samples. However, no melatonin was detected in other plant samples. Melatonin is a naturally occurring compound found in small quantities in various foods, including edible plants. Researchers have detected melatonin in different plant parts such as flowers, fruits, stems, roots, leaves, and seeds. For instance, Chen et al. [31]

reported that the melatonin concentration in dried goji berries and white mulberry leaves was measured at 530 and 1510 ng/g, respectively. In a study conducted by Kolar and Malbeck [32], it was reported that the melatonin amount in blackberry was measured as 21 pg/g. In *Prunus* species, it was reported that melatonin content in ripe fruits of *Prunus cerasus* and *Prunus avium* species ranged from 2.06 to 13.46 ng/g [33, 34] and 0.01–20 ng/g [33-35], respectively. Variations of the melatonin contents in different plants are reasonable relative to the physical and chemical characteristics of plant samples. The melatonin concentrations in plants are closely influenced by factors such as extraction methods, species, harvesting time, growing environment, varieties, and cultivated methods. Regarding the melatonin content of plants, considerable differences exist in the course of developmental processes, too. The various environmental or biotic stress factors lead to changes in melatonin. In conclusion, the identification of biologically active molecules in edible vegetables and fruits, and herbaceous plants used in traditional medicine will shed light on new studies.

5 AUTHOR CONTRIBUTIONS

Hypotesis: S.E., Y.U.; Design: E.Y., A.M., S.E.; Literature review: Y.G., A.T.T.; Data Collection: Y.G., A.T.T, A.M., E.Y; Analysis and/or interpretation: Y.U., Y.G., A.T.T., A.M., E.Y., Z.M.; Manuscript writing: Y.U., S.E.

6 CONFLICT OF INTEREST

Authors declare that there is no conflict of interest. Jsldbfkjs

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