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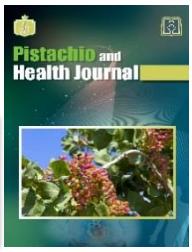

ORIGINAL ARTICLE

Pistacia atlantica as an inexpensive source of melatonin

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Background: Melatonin is involved in regulation of circadian rhythm and alleviation of sleep disorders, such as insomnia due to jet lag and shift work, as it plays a major role in synchronization of the sleep/wake cycle

Materials and methods: This study is devoted to extraction and determination of melatonin in *Pistacia atlantica* using an ultrasound-assisted solid-liquid extraction (SLE) combined with UV-Vis Spectroscopy. The volume and type of extraction solvent, sonication time, and extraction temperature on the extraction efficiency were optimized.

Results: Under optimum conditions, use of 30 mL of ethanol and 30 min sonication time caused to achieve the limit of detection (LOD) of 0.047 ppm. The method accuracy was confirmed according to the calculation of recovery using standard addition method that, exhibited the successful applicability of the present method for real sample analysis. In addition, the obtained results were compared to those using gas chromatography/Mass spectroscopy (GC/MS). Combination of SLE and UV-Vis leads to greater sensitivity and lower cost for accurate and repeatable extraction of melatonin from *Pistacia atlantica* with acceptable recovery and RSD% (1.04%).

Conclusion: Therefore, we efficiently extracted and purified of melatonin from an inexpensive source (*Pistacia atlantica* fruit). The extracted melatonin can be replaced synthetic drugs.

Keywords: Melatonin; Solid-liquid extraction; Ultrasound-assisted extraction

1. Introduction

Melatonin is a type of indolamine (N-acetyl-5-methoxytriptamine) that is natural in various vegetables and fruits [1, 2]. Melatonin is involved in the regulation of the circadian rhythm and the alleviation of sleep disorders such as insomnia due to the jet lag and the shift work, since it plays a major role in the synchronization of the sleep-wake cycle [3, 4]. Other roles of melatonin include the protecting of DNA against the damage that may be caused by free radicals. Melatonin has been considered to have effective antioxidant and anti-inflammatory properties [5, 6]. Melatonin mitigates neurodegenerative diseases (Alzheimer and Parkinson) [7] and plays the role of an anticancer agent [8, 9]. In addition, melatonin has been used to treat depression, contraception, and fight AIDS ravages and aging. Therefore, it is important to know more about the physiological and pharmacological effects of this hormone. In addition, its chemotherapeutical, immunological and toxicological aspects are important [10]. Due to the low levels of melatonin in samples, the dearth of analytical methods and the complexity of the biological matrices, it is necessary to be able to accurately extract and quantify the levels of melatonin present in foods.

Pistacia atlantica is one of the wild species of pistachios that is of the highest distribution rate and is considered as an Iranian-Turanian species being distributed from southwest Asia to northwest Africa [11]. The main origin of this plant is the Iranian Plateau. In Iran, three subgroups of *Pistacia atlantica* are found. The subgroup of cabulica is distributed in the center and Southeast of Iran. The subgroup of muticais distributed in the northwest, center, northeast, east and south, and the subgroup of kurdica is distributed in the west, southwest and a specific region in the center of Iran. The area of *Pistacia atlantica* forests in Iran is 2 to 2.5 hectares [12]. *Pistacia atlantica* grows in different regions of Iran such as Zagros, Kordestan, Lorestan, Khuzestan, Fars, Baluchestan, Kerman, Khorasan and Yazd [13]. *Pistacia atlantica* is of invigorating and stimulant properties. It is used to cook local foods and treat stomach ulcers and anemia. The fruit of *Pistacia atlantica* is used as nuts, and its oil is extracted for medicinal applications. The amount of the oil present in *Pistacia atlantica* fruit is about 25 to 30 percent. [14] The oil of *Pistacia atlantica* is a rich source of Tocopherol that is an important component of vegetable oils, plays an antioxidant role and is active as vitamin E [15].

One of the important analytical steps is the extraction process in the separation and recognition of compounds from solid samples prior to analytical measurements. Ultrasound is a kind of radiation nowadays applied in developing practical techniques in the industry, engineering, medicine, and chemistry. Ultrasound-assisted extraction (UAE) is recognized as an efficient approach utilized for the extraction of compounds from solid samples, due to its simplicity, speed and inexpensiveness. Particularly, analytical chemists have widely applied the UAE method for the extraction of various compounds such as melatonin

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from different vegetables and fruit matrices, including *Pistacia Varieties* [16] and rice grains [17]. Many methods have so far been utilized for identifying melatonin, including GC/MS [18-20], UV–Vis spectroscopy [21, 22], capillary electrophoresis [23] and electrochemical detection [24]. Among the methods mentioned, the UV-Vis technique is simple and inexpensive. Therefore, in the current study, researchers have proposed the ultrasonic-assisted solid-liquid extraction method (SLE) for the extraction of melatonin, prior to utilizing the UV-Vis spectrophotometer.

2. Materials and Methods

2.1. Reagents and instrumentation

Analytical grade methanol, ethanol and acetonitrile were supplied from Merck (Damstadt, Germany). Melatonin was purchased from Sigma-Aldrich (Steinheim, Germany). *Pistacia atlantica* fruits were obtained from Baft in Kerman Province, Iran.

The gas chromatography/mass spectrometry (GC/MS) analysis was carried out on a Hewlett–Packard HP 6890 Gas Chromatograph (Palo Alto, California, USA) connected with a mass detector HP 5793, using an HP-1 column (30 m×0.25 mm, film thickness of 0.25 µm). UV-Vis Spectrophotometer analyses were conducted on Specord210 plus (Jena, Germany). The extraction process was performed using a Sonorex RK255 ultrasonic water bath obtained from Bandelin (Berlin, Germany).

2.2. The Ultrasound-assisted solid-liquid extraction of melatonin from *Pistacia atlantica* (UASLE)

Five tenths of a gram of the milled *Pistacia atlantica* fruit were weighed and mixed with 50 mL ethanol in a round-bottom flask. The mixture was placed and sonicated in an ultrasonic bath for 30 minutes. The pre-treated mixture solution was then transferred to a test tube where it was centrifuged for 10 minutes at 4000 rpm. Afterwards, 250 µL of the supernatant separated was transferred to a 5 mL volumetric flask, and ethanol was added to the volume. Next, the absorbance of the solution was measured by the UV-Vis spectrophotometer.

2.3. GC/MS conditions

The experimental conditions were as follows: the oven temperature was programmed from 40 °C (1 min) to 250 °C (60 min) at 3 °C/min; the injector and detector temperatures were 250 °C and 230 °C, respectively; the carrier gas helium 99.999% was at a flow rate of 1 mL/min. The mass spectrometer was operated at 70 eV with the mass range of 40–350 amu, and the scan time of 1 second.

3. Results and Discussion

3.1. The identification of melatonin using GC/MS

The melatonin peak in the chromatogram of the extract obtained was distinguished by comparing its retention time with the one in the chromatogram of a standard solution of melatonin. The mass spectrum of the peak assigned was in good agreement with those of an authentic sample in NIST MS library V.2.0.1 (Fig. 1), confirming the presence of melatonin in the extract of *Pistacia atlantica*.

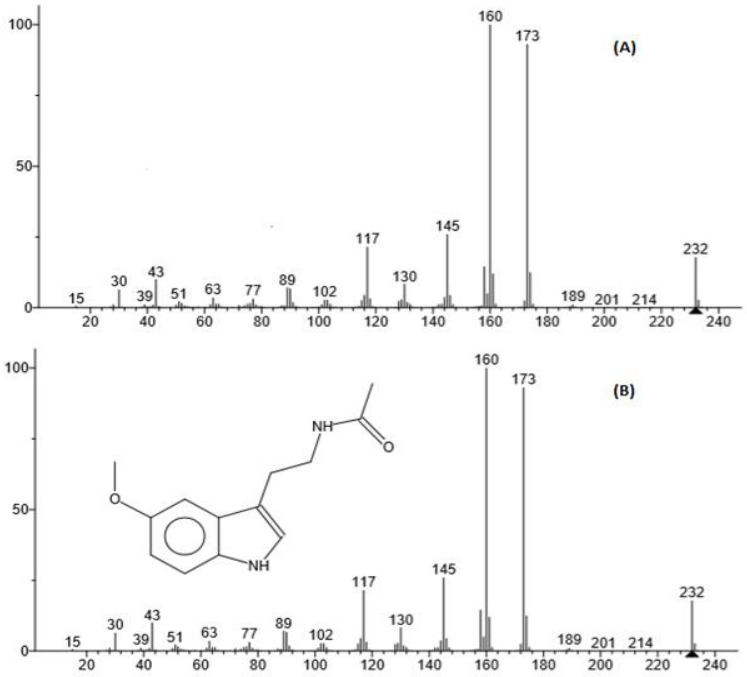

Fig. 1. The mass spectra of melatonin in the *Pistacia atlantica* extract (A) and standard melatonin solution (B).

3.2. The optimization of the UA-SLE procedure

Many factors can determine the efficacy of a solid-liquid extraction, including the type and volume of the extracting solvent, the ultrasonic time and the temperature. Therefore, it is useful to study these factors in the current research. The UV-Vis peak related to the melatonin standard is given in Fig. 2.

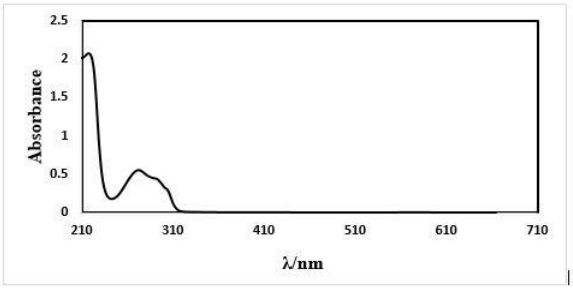

Fig. 2. The UV-Vis peak of standard melatonin solution

3.2.1. The effect of the extracting solvent

The type of the extracting solvent, considered as a significant factor, controls the melatonin extraction efficiency. Ethanol, methanol and acetonitrile were selected as the potential extracting solvents in this study. They were examined under similar conditions and methods. Fig. 3 presents a comparison between these solvents. As it is demonstrated, the use of ethanol as an extracting solvent results in the highest amount of the melatonin extracted.

3.2.2. The effect of the volume of the extracting solvent

To optimize the volume of the extraction solvent, the extraction procedure was followed on similar samples applying different volumes of ethanol (5 to 50 mL). As Fig. 3 demonstrates, the absorbance intensity of the extracted melatonin increased with the increase in the ethanol volume up to 25 mL, and then it remained fixed with the further increase up to 50 mL. Therefore, 25 mL of ethanol was chosen as the optimal volume.

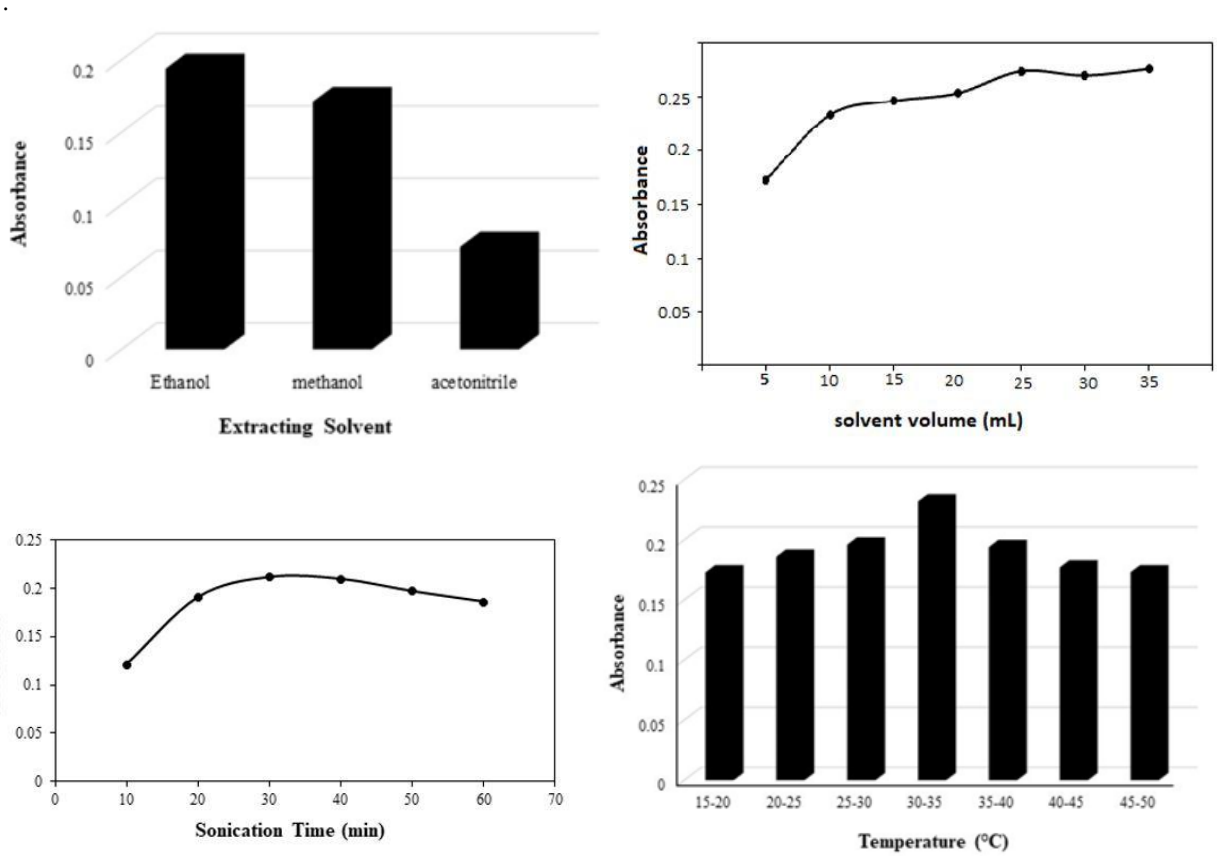

Fig. 3. Effects of the type and volume of the extracting solvent, sonication time and temperature on the absorbance of the extracted melatonin.

3.2.3. The effect of the ultrasonication time

The extraction time is a factor that influences the extraction efficiency. In the present protocol, the extraction of melatonin occurs during the ultrasonication phase. Hence, to investigate the time of the extraction process, different sonication times within the range of 10-60 minutes were used for the extraction of melatonin under similar conditions. Fig. 3 shows the results obtained. As it is depicted in Fig. 3c, the extraction efficiency increased with the increase in the sonication time up to 30 minutes, and then it remained almost constant.

3.2.4. The effect of the extraction temperature

The extraction processes were completed at different temperatures from 10 ºC to 50 ºC for 30 minutes. The highest absorbance intensity was observed when melatonin was extracted at 30-35 ºC (Fig. 3). Accordingly, next extraction experiments were carried out at 30 ºC as the optimum temperature.

3.3. Analytical performance

To construct a standard addition calibration curve, various volumes of the standard solution of melatonin (400-1000 uL) were added to 250uL aliquots of the *Pistacia atlantica* extracted and finally, the volume of each solution amounted to 50 mL. Afterwards, the absorbance of the samples prepared was measured. Using the absorbance plot against the melatonin concentration, the concentration of melatonin in the *Pistacia atlantica* extract was calculated at 10.51 ppm. The calibration curve was linear in the concentration range of 0.1-100 ppm with the correlation coefficient of (R^2) 0.997. The limit of detection (LOD) for the detection of melatonin was 0.047 ppm (calculated from $3S_b/m$, S_b: the standard deviation of blank, m: the slope of the calibration curve). The relative standard deviation (RSD) of the method for the determination of 0.5 ppm melatonin was calculated at ±1.04%, indicating the satisfactory repeatability of the method proposed.

The amount of melatonin in the *Pistacia atlantica* extract was quantified using UV-Vis spectroscopy by comparing the peak area of melatonin in the extract sample with those of the standard melatonin solutions. Spiked samples were also analyzed to confirm the accuracy of results. The corresponding results are given in Table 1. As it can be seen, recovery values were 89.0% and 91.2% indicating the high accuracy of the method developed. Moreover, the total amount of melatonin in *Pistacia atlantica* was found to be 0.29 mg/g.

Table 1. Determination of melatonin in the extract using UV-Vis spectroscopy

Sample	Spiked (ppm)	Found[a] (ppm)	Recovery (%)
Pistacia atlantica extract	-	10.51±0.02	-
	0.8	11.24±0.12	91.25
	1.0	11.40±0.04	89.0

[a] Mean±standard deviation

3.4 The comparison of the present study with some other methods

The comparison results of the method proposed with some techniques reported [25-27] for the purification and determination of melatonin are given in Table 2. As it is demonstrated, this method presents acceptable figures of merit, the low detection limit, and the good linear range in comparison with other methods.

4. Conclusion

In the present research, the ultrasound-assisted extraction of melatonin from *Pistacia atlantica* fruit and its method of identification, purification and quantification using GC/MS were proposed. The extraction condition was also optimized. Considering the extraction yield, the optimal conditions included 30 mL ethanol as the extracting solvent, the extraction temperature of 30-35°C, the extraction time of 30 minutes and the centrifuge time of 10 minutes. Under optimized conditions, the melatonin content in *Pistacia atlantica* was obtained at 10.51 ppm. Concerning the various medical benefits of melatonin, its presence in Pistacia atlantica makes this nut a delicious source of melatonin for people.

Table 2. Comparison of the proposed method with some reported techniques.

Method	Linear range (ppm)	LOD (ppm)	RSD%	Ref.
Molecularly Imprinted Sensor	0.1-23	0.001	1.3	(25)
capillary electrophoresis	1-10	0.54	1.6	(26)
Melatonin in root	0.1-50	0.01	2.6	(27)
SLE	0.1-100	0.047	1.04	This work

Conflict of interest

The authors declare no conflicts of interest.

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