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RESEARCH ARTICLE

MELATONIN STABILITY AT DIFERENT STORAGE CONDITIONS AND DURING THEULTRASOUND–ASSISTED EXTRACTION (UAE)

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ABSTRACT

The stability of melatonin in different storage conditions and under ultrasound-assisted extraction has been studied.This work aimed on the analysis of the melatonin stability variation with time and under the light, air and temperature effects, in order to determine the best recommended storage conditions. The results showed that melatonin has an acceptable stability in all tested storage conditions: Darkness at 25ºC, darkness at 50ºC, light at 25ºC and light at 50ºC until day 13. However, the samples kept at 25ºC in light and in darkness without air protection; which degrades strongly from the beginning, to reach more than 41% on the sixth day and more than 66% on the fifteenth day. Under the ultrasound-assisted extraction conditions, e.g. the presence of air, incidence light and high temperature (50 ºC), the results showed that the melatonin is stable and not degradable at higher temperature.

INTRODUCTION

Melatonin, or N-acetyl-5-metoxytryptamin, is a neurohormone primarily produced by the pineal gland in vertebrates and follows a circadian pattern. Thus, melatonin is widely distributed in the animal kingdom. It can be found in some foods such as walnuts, rice, tomato, strawberry, almond, mustard and sunflower, and it also produced as secondary metabolite in plants.Melatonin involved in the regulation of circadian rhythm (Srinivasan *et al.,* 2010) and the alliviation and reduction of sleep disorders, such as insomnia due to jet-lag and shift work as it plays a major role in in the synchronisation of the sleep/ wake cycle (Buscemi *et al.,* 2004 and 2005; Murch*et al.,* 2000). Furthermore, the melatonin displayed potent anti-oxidative and anti-inflammatory properties (Reiter *et al.,* 2000 and 2005; Tan *et al.,* 2003). In addition, some of the protective effects of melatonin in ocular diseases have also been described (Siu, Maldonado *et al.,* 2006). Several studies reported the anticarcinogenic properties of melatonin (Hardeland *et al.,* 2006). Melatonin mitigates neurode generative diseases, such as Alzheimer´s and Parkinson´s diseases (Wang, 2009). It is reported that melatonin might be used in countering the levels of free radicals that generated by metabolic activities.

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Likewise, the direct exposure to sunlight or UV light that promotes photo-oxidation could also have an influence on the biosynthesis of melatonin in plants, increasing its production levels (Murch *et al.,* 2000; Tan *et al.,* 2007).Studies related on the role of melatonin in plant, in biochemical and molecular aspect of plants are limited by a number of factors, including less efficient detection methods, low concentrations, andinsufficient experimental devices. The presence of melatonin in food samples can be determined and confirmed by different extraction procedures, together with efficient analytical methods. Melatonin can be detected by several methods, such as immunological techniques, Radioimmunoassay (RIA) (Manchester *et al.,* 2000), Enzyme-Immunoassay (EIA) (De la Puerta *et al.,* 2007), chromatographic techniques with different detectorsas: fluorimetric (Iriti *et al.,* 2006), GC/MS (Gonzalez Gomez *et al.,* 2009), MS/MS (Cao *et al.,* 2006 ; Rodriguez Naranjo *et al.,* 2011), and chemiluminescent techniques (García Parilla *et al.,* 2009). The chromatographic techniques are more economical and time efficient. Indeed, in this technique, the derivatization of the sample is not required prior to analysis. The most reviewed HPLC methods used a reverse phase columns (e.g. RP18 or RP8) for melatonin separation. The fluorescence detectors (FD) used in HPLC techniques were found to be sensitive and versatile to quantify melatonin in food samples and also gave low limits of melatonin detection and its quantification. (García-Parilla *et al.,* 2009).

Several extraction methods of melatonin from vegetable and food samples have been reported, including ultrasound-assisted extraction (Cao *et al.,* 2006; Rodriguez Naranjo *et al.,* 2011), liquid-liquid extraction (Reiter *et al.,* 2005) and solid phase extraction (Chen *et al.,* 2003; García Parilla *et al.,* 2009).However, the most of those methods were used as a prior step for chromatographic analyses.The application of ultrasound as a laboratory based technique for assisting extraction from plant material is widely used. Several studies have been reported in the extraction of metabolites from original plant (Knorr 2003), flavonoids from foods using a range of solvents (Zhang, Xu and Shi 2003) and bioactive metabolites from herbs (Vinatoru, 2001). The objective of thepresent work is (i) to provide evidences on how the quality of melatonin stability varies during time, and under the influence of several factors such as temperature, air and light, and (ii) to establish the optimum storage conditions. The stability during time of extracted samples using ultrasonic-assisted extraction at 25 and 50ºC, and in the presence and absence of light is investigatedby HPLC-FD.

MATERIALS AND METHODS

Reagents

Melatonin standard is purchased from Sigma-Aldric™ (Somaprol, s.a.r.l). Methanol (HPLC grade) is purchasedfrom Fluka, glacial acetic acid for analysis ispurchased from Riedel dehaën. Solutions are prepared bydiluting with pure water.

Melatonin sample preparation

0.2 mg.$L^{-1}$of melatonin standard is prepared in pure water.
The calibration curve islinear over the following concentration ranges:0.02 and 0.16 mg.L^{-1} of melatonin.

Storage conditions

Table 1 shows the assayed storage conditions and their related codes.

Table 1. Storage conditions of melatonin and codes used to identify them

Storage conditions	Temperatures Tº (ºC)	Air	Light
D25	25	No	No
D50	50	No	No
DA25	25	Yes	No
L25	25	No	Yes
L50	50	No	Yes
LA25	25	Yes	Yes

Melatonin samples stored under indicated conditions were analyzed by HPLC after 2, 6, 8, 10, 13 and 15 days; and the melatonin levels are compared with initial melatonin levels (*i.e.,* before the storage).

UAE conditions

To study the influence of variables that could influence the stability of melatonin standard using ultrasound-assisted extraction, different extractions conditions were assayed.

Table 2 shows the assayed UAE conditions and their related codes.

Table 2. UAE condition solutions of melatonin and codes used to identify them

UAE conditions	Temperature (ºC)	Darkness	Light
UAE25	25	No	Yes
UAED25	25	Yes	No
UAE50	50	No	Yes
UAED50	50	Yes	No

The extraction protocol used melatonin standard diluted in pure water (5mg.L^{-1}), the samples were extracted each 5min for 60 minutes.

Determination and extraction of melatonin

HPLC-FD

Chromatographic analyses were carried out on an Alliance® System HPLC 2695 with pump system (Waters 600) and fluorescence detector. The column used in this study was Symmetry® C18 (5µm); 4,6mmx150mm from Waters. Millennium chromatographic software was used for HPLC control and peak integration. An isocratic elution was used with two mobile phases: phase A (2% acetic acid and 8% methanol in water) and phase B (2% acetic acid and 8% water in methanol). Isocratic elution was used applying 50/50 (A: B) at a flow rate of 0.5 ml/min; and injection volume of 20µL. For the fluorescence detector; the fixed conditions were as follows: an excitation wavelength of λ= 280 nm and an emission wavelength of λ= 310 nm. HPLC mobile phases were first degassed in an ultrasonic bath and were filtered through a 0.45 µm membrane before analysis with HPLC-FD. All extractions were performed in duplicate.

Ultrasound-Assisted Extraction (UAE)

The Ultrasound-Assisted Extraction instrument is composed of a high intensity ultrasonic probe 50/60 kHz, model UP 200S (Dr.Hielsher Company, Germany). The probe was applied into the liquid samples. The samples were immersed in a water bath coupled to a temperature controller (thermostatic bath Frigiterm, J.P Selected, Barcelona, Spain)

RESULTS AND DISCUSSION

Storage stability

Temperature, light, air and time are the four variables that thought likely to influence the stability of melatonin during storage period.

To evaluate the effect of temperature, the results obtained are compared to store solutions of melatonin in two different temperatures: 25ºC and 50ºC as showed in Table 1. Those solutions were analyzed for 15 days using different new vials for each control point. The experimental data showing the effects of the storage conditions of melatonin standard are presented in Figures 1 &2.

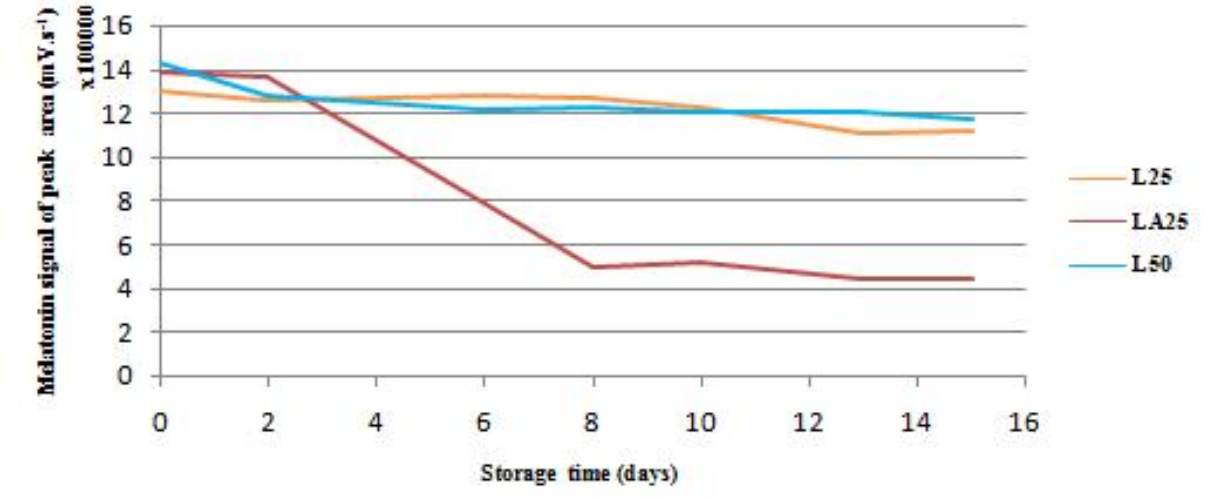

Figure 1. Time evolution of the samples stored in four different temperatures in the presence of light L25 & L50ºC and in the presence of light with oxygen at room temperature LA25ºC

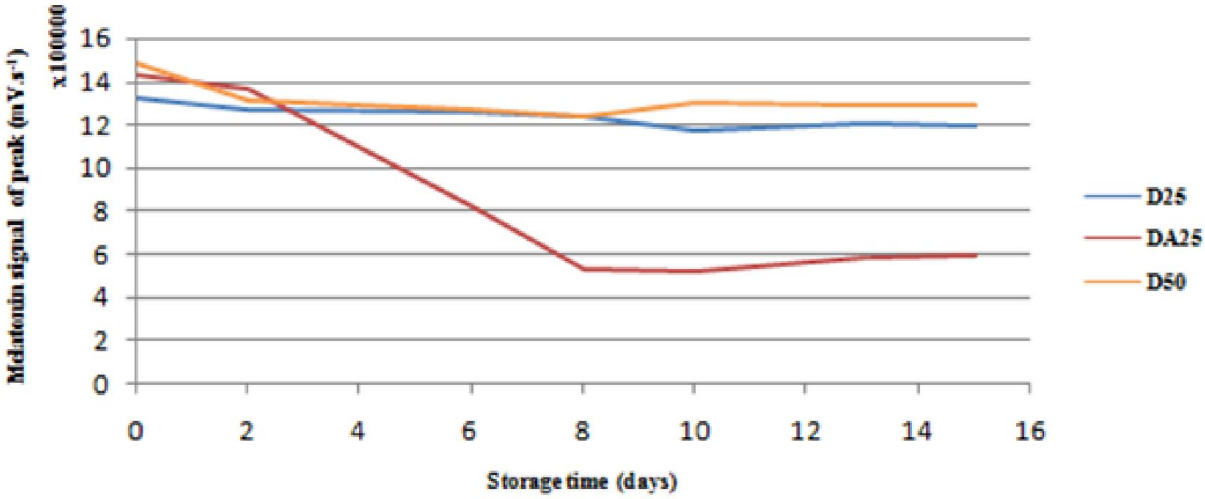

Figure 2.Time evolution of the samples stored in four different temperatures without light D25 & D50ºC and without light & air at room temperature DA25ºC

Figure1 shows the results of storage conditions of melatonin in light at three different storage locations: L25; L50 and LA25.

The results obtained demonstrate that the concentration of melatonin standard was not significantly decreased in all storage conditions until day thirteen, *i.e.* no significant degradation up to day thirteen. After this day, it has been registered a high degradation reaching up to 22% in L25 and L50. For the samples that are kept at room temperature in the presence of light and air LA25, it was observed that the concentration of melatonin was significantly decreased from the sixth day of storage time, and reaching more and less 43% and suffers clear degradation on day fifteen (68%). Therefore, the temperature cannot be considered as animportant variable for melatonin degradation, at least using up to room temperature. Therefore, it can be concluded that melatonin solutions can be maintained up to thirteenth days at 25ºC without significant degradation.

By comparing the stored samples in light without air L25 and L50 with the samples stored in light in contact with air LA25; no differences were recorded between the store samples and melatonin stability remained stable until day sixth. Regarding samples stored in light with air, the melatonin solutions showed melatonin degradation starting at day 6, then reaching up to 43%, more intense degradation was observed however on day 15 reaching up to 68%. Therefore, the presence of light and the oxygen clearly increases the melatonin degradation. So, air is an important variable to be considered regarding melatonin stability. It has to be noted that no extra air was added to the samples vial through the storage, therefore it can be supposed that the initial fast degradation is due to the air available at the beginning, then decreasing because no more air was added into the vial to maintain a longer stability of samples. The effect of darkness as another variable on the stability of melatoninhas been evaluated by considering the same three different storage locations: D25; D50 and DA25. The obtained results are shown in Figure 2.

As can be seen in Figure 2, there are no differences between the results obtained for the samples stored in darkness without air at any point, and very similar results were obtained until day 13, except the samples stored at room temperature and in contact with air which start to degrade from the day sixth (41%) and more intense degradation was observed on the day fifteen (66%). Comparing the storage conditions of samples stored in light presented in Figure 1 with the samples stored in darkness presented using light or light protected conditions at any control point, very similar results were obtained during storage time. Therefore, light is not an important variable to be considered regarding melatonin stability. From Figures 1 & 2, one can conclude that cannot preserved melatonin samples in darkness and in light and without air for more than 13 days, and in contact with air for more than 6 days.

Stability analysis by UAE

Because the air contact makes the melatonin degradation faster than in the regular storage conditions and due to during the ultrasonic-assisted extraction, there is a large amount of air continuously introduced in the sample.

It was thought that it is interesting analyzing the melatonin stability through this extraction process. The influence of the temperature and sonication time (5-60 min) with and without light on the melatonin samples was investigated. During this type of extraction air cannot be avoided, in fact usually full air saturation can be obtained.Two series of samples at room temperature (25ºC)were run: the first in the presence of light (UAE25), while the second in its absence (UAED25). Usually, the temperature for the extraction liquid increases during extraction due to the ultrasound energy released in the media, and then a higher temperature was also assayed in two different extraction conditions, *i.e.* regular light (UAE50) and protected light (UAED50).

All samples were prepared in water; and the temperature was kept constant by a cooling/heating coil immersed into the bath. The sonication period was up to 60 min; and samples were collected every 5min. Figures 3 & 4 show the results for both extractions at 25ºC and at 50 ºC.

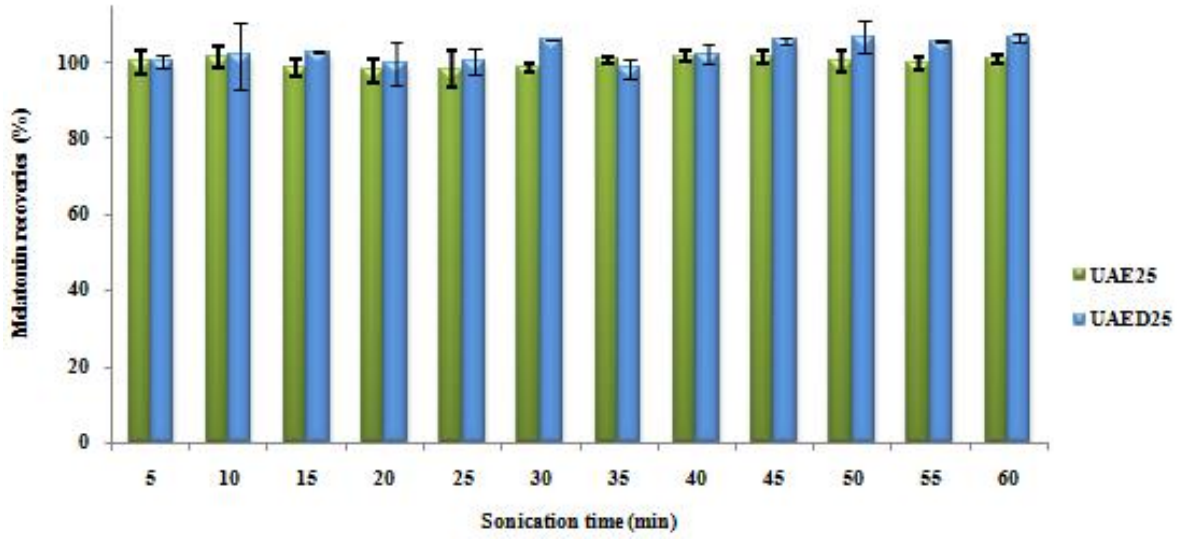

Figure 3. Recovery results after treatment by ultrasound with and without light at 25ºC (UAE 25 & UAED 25)

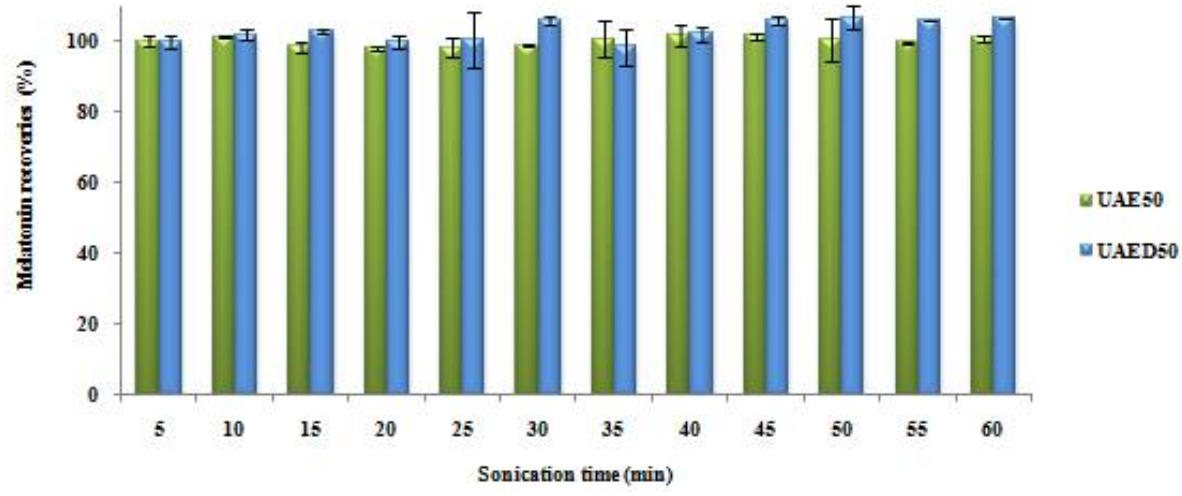

Figure 4. Recovery results after treatment by ultrasound with and without light at 50ºC (UAE 50 & UAED 50)

As can be seen in Figure 3, melatonin was not degraded under ultrasound assisted extraction conditions for 60 min at 25º C. Previously (Fig. 2) the synergetic effect between air and light was observed during storage conditions study, however for ultrasound assisted extraction conditions, no effect was found for light when a high oxygen level was found in the sample, at least for 60 min there is no significant degradation. Extractions at high temperature (50ºC) were run also for onehour (Figure 4). No differences were found for melatonin solutions under extraction conditions with or without light protection at any extraction time. Additionally, no differences were found after onehour of extraction conditions. Therefore, melatonin was stable under ultrasound-assisted extraction conditions at 50ºC. Usually, this temperature is near the highest values regularly used for UAE because higher temperatures are near the boiling point of many solvents, then ultrasound efficiency declines because of surface tension is lower, which in turn cause the damping of the shock waves.

Conclusion

It has been shown that melatonin solutions are stable in storage conditions tested both in the presence and absenceof light; except the samples preserved with air. Therefore, the extracts can be stored using condition of air only for 6 days.It has been also found the stability of this compound in the ultrasonic extraction conditions in two different temperatures, both in the presence of light or in darkness.This showedthat light do not affect the stability of melatonin during the sonication time, neither at higher temperature (50ºC). Making it feasible to raise the development of an extraction method based on this methodology for recovery of melatonin.

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REFERENCES

Buscemi, N., Vandermeer, B., Hooton, N., Pandya, R., Tjosvold, L., Hartling, L. *et al.* 2005.The efficacy and safty of exogenous melatonin for primary sleep disorders a meta-analysis. *Journal of General Internal Medicine,* 20 (12).1151-1158.

Buscemi, N., Vandermeer, B., Hooton, N., Tjosvold, L., Pandya, R., Hartling, L., Baker, G., Vohra, S., Klassen, T. 2004. Melatonin for Treatment of Sleep Disorders, Evidence Report/Technology Assessment of Agency for Healthcare Research and Quality, 108, p 1-7.

Cao, J., Murch, S.J., O'brien, R. and Saxena, P. K. 2006. Rapid method for accurate analysis of melatonin, serotonin, and auxin in plan samples using liquid chromatography-tandem mass spectrometry. *J. Chromatography A,* 1134 (1-2), 333-337.

Chen, G., Huo, Y., Tan, D., Liang, Z., Zhang, W. and Zhang, Y. 2003.Melatonin in chinese medicinal herbs. *Life Science,* Vol.73.

De la Puerta, C., Carrascosa-Salmoral, M.P., García-Luna, P.P., Lardone, P.J., Herrera, J.L., Fernandez- Montesinos, R., Guerrero, J.M. and Pozo, D. 2007. Melatonin is a photochemical in olive oil. *Food Chemistry,* 104, 609-612.

Gonzalez gomez, D., Lozano, M., Fernandez Leon M. F., Ayuso, M. C., Bernalte, M. J. and Rodriguez, A. B. 2009.Detection and quantification of melatonin and serotonin eight sweet cherry cultivars (Prunusavium L). *European Food Research Technology*, (229), 223-229.

Hardeland, R., Pandi-Perumal, S.R., andCardinali, D.P. 2006. Melatonin.*The International Journal of Biochemistry and Cell Biology,* 38(3), 313-316.

Iriti, M., Rossoni, M. and Faoro, F. 2006. Melatonin content in grape: myth or panacea? *Journal of the Science of Food and Agriculture,* 86, 1432-1438.

Knorr, D. 2003. Impact of non- thermal processing on plant metabolites.*Journal of Food Engineering,* 56, 131-134.

M. Carmen García Parilla M.C., Emma Cantos and Anna M. Troncoso. 2009. Analysis of melatonin in foods. *Food Composition and Analysis,* 22:177-183.

Murch, S.J., Krishna Raj, S. and Saxena, P. K. 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in vitro regenerated St. Jhon´swort (Hypericum

Reiter, R.J., Calvo, J.R., Karbownik,M., Qi,W., and Tan,D. X., 2000. Melatonin and it relation to the immune system and inflammation. *Annals of the New York Academy of Sciences,* 917, 373-386.

Reiter, R.J., Manchester, L. C. and Tan, D.X. 2005. Melatonin in walnuts: Influence of LevelsMelatonin and Total AntioxidantCapacity of Blood, Nutrition, 21, p920-924.

Rodriguez-Naranjo, M.I., Gil-Izquierdo, A., Troncoso, A.M., Cantos-Villar, E.and García-Parilla, M.C.2011.Melatonin is synthetised by yeast during alcoholic fermentation in wines. *Food Chemistry,* 126, 1608-1613.

Siu, A.W., Maldonado, M., Sanchez-Hidalgo, M., Tan, D. X.andReiter, R.J. 2006. Protective effect of melatonin in experimental free radical-related ocular diseases. *Journal of Pineal Research,* 40(2).101-109.

Srinivasan, V., Singh, J., Pandi-Perumal, S.R., Brown, G.M., Spence, D. W.andGardinali, D.P. 2010. Jet lag, circadian rhythm sleep disturbances, and depression: The role of melatonin and its analogs. *Advances in Therapy,* 27(11), 796-813.

Tan, D.X., Hardeland, R., Manchester, L.C., *et al.,* 2003.Mechanistic and comparative studies of melatonin and classic antioxidants in therms of their interactions with the ABTS cation radical. *Journal of Pineal Research,* 34(4), 249-259.

Tan, D.X., Manchester, C., Di Mascio, P., Martinez, G.R., Prado, F.M., and Reiter, R.J. 2007. Novel rhythms of N1-acetyl-N2-formyl 5methoxykynuramine and its precursor melatonin in water hyacinth: Importance for phytoremediation. *The FASEB Journal,* 21, 1724-1728.

Vinatoru, M. 2001. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry,*8(3), 303-313.

Wang, X. 2009. The antiapoptotic activity of melatonin in neurodegenerative diseases. *CNS Neurosci. Ther.,* 15, 345-357.

Zhang, R., Xu, Y., and Shi, Y. 2003.The extracting technology of flavonoids compounds. *Food and Machinery,* 1, 21-22

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