

Garcinia mangostana L. Fruit Peel: A Novel Source of Melatonin

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ABSTRACT

Background: Melatonin (N-acetyl -5- methoxy-tryptamine) is a mammalian neuro hormone, with numerous biological functions found in animals, plants, microorganisms and one of the extensively used dietary supplements. Phytomelatonin offer a great potential as Nutraceutical in pharmaceutical industry since it is safe with no adverse effects. However, it is reported to be found in negligible quantities. Melatonin is found in all parts of the plant and fruit peels which are considered as waste can be excellent source of Melatonin. **Materials and Methods:** The objective of the study was to screen fruit peels for phytomelatonin. Initially a quantitative test of *Garcinia mangostana*, *Citrus aurantifolia*, *Punica granatum*, *Citrullus lanatus*, *Artocarpus heterophyllus*, *Citrus lemon*, *Cucumis melo*, *Selenicereus undatus*, *Musa sp.*, *Ananas comosus*, *Borassus flabellifer*, *Litchi chinensis* and *Citrullus sp.* fruit peel methanolic extract had positive Reaction with Human Melatonin ELISA Kit and the confirmatory test using HPLC were done **Results and Discussion:** Further confirmatory and quantification of melatonin using ELISA Melatonin Kit revealed *Garcinia mangostana* fruit peel had highest melatonin with 2.2 ng/g peel, when compared to other fruit peels. The HPLC analysis of methanolic extract had a retention time of 1.315 similar to that of standard melatonin. **Conclusion:** From the above study it is concluded that fruit peels of *Garcinia mangostana* is considered as agricultural waste could be potential source of Melatonin which can used as nutraceuticals for a better Human health.

Keywords: *Garcinia mangostana*, Fruit peel, Phytomelatonin, Neurohormone, Biological properties.

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INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone concerned with circadian (Ng *et al.*, 2020) timings, sleep (Reiter 2003), a mild tranquilizer, maintain the core body temperature (Claustrat *et al.*, 2005), most effective antioxidant (Ferlazzo *et al.*, 2020) and anti-aging component (Zisapel *et al.*, 2018). It can reduce the risk of stroke and coronary heart diseases (Sewerynek 2002), immune-modulating (Vijayalaxmi *et al.*, 2002), anti-inflammatory, can cure Alzheimer's diseases (Cardinali *et al.*, 2002), Osteoporosis (Erdem *et al.*, 2014), Neurodegenerative (Tamtaji *et al.*, 2019) disease and prevent cancer (Chen *et al.*, 2019). Melatonin is also effective against viral diseases especially COVID-19 (Zhou *et al.*, 2020) and hence used extensively as a dietary supplement. According to the reports of American Psychiatric Association (APA), most adults suffer from insomnia and hence the use of synthetic melatonin has increased tremendously after COVID-19 (Tan and Reiter, 2022). The global

production of synthetic melatonin was around 1.3 billion USD in 2019 and projected to increase by more than 10% by 2024. About 50% of synthetic melatonin is used for medical purposes and the remaining used in chemical and industrial purpose. Synthetic melatonin produced by chemical synthesis from tryptophan derivatives produce toxic by-products that sometimes Cause disease like Eosinophilia Myalgia Syndrome (Williamson *et al.*, 1998). Melatonin produced using phthalimide (He *et al.*, 2003) and allylamine (Verspui *et al.*, 2000) are dangerous and toxic. Synthetic and commercial melatonin can be replaced with the natural source of Melatonin (Arnav and Hernandez-Ruiz, Cipolla-Neto and Amaral, 2018). It was believed that Melatonin was found only in humans and Mammals for past two decades. Recently, Melatonin was discovered in Plants and other living organisms (Reiter *et al.*, 2007, Perez- Llamas *et al.*, 2020, Altaf *et al.*, 2021).

Melatonin in Plants is called Phytomelatonin which was discovered in the 1995 (Perez- Llamas *et al.*, 2020, Altaf *et al.*, 2021). Phytomelatonin is a broad antioxidant (Arnao and Hermendez- Ruiz, 2020). Phytomelatonin is present in numerous flowering plants, especially in medicinal herbs in all parts of the plants like root, stem, leaf, flower, fruit and seeds (Zohar *et al.*, 2011, Bhattarcharjee and Dey, 2018) However, it is found in



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negligible quantities. When compared to synthetic melatonin, Phytomelatonin is better anti-inflammatory and antioxidants (Kukula-Koch *et al.*, 2021) Although phytomelatonin can replace synthetic melatonin, detection of Phytomelatonin is not an easy process (Arnao 2014). Conventionally it is detected through Fluorescence and Radiolabeling Techniques. These techniques are quite expensive and requires expertise personal, hence search for alternative plant source and detection method for Phytomelatonin is inevitable.

Garcinia mangostana L. called “Queen of the Fruits” has an excellent antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial and antiviral properties and rich in alpha, beta, gamma Mangostins, Garcinone E and Gartanin. It is used for treatment of abdominal pain, diarrhea, dysentery, infected wounds, suppuration and chronic ulcer (Pedraza- Chaverri *et al.*, 2008). Earlier *Garcinia mangostana* peel was reported to contain ample amount of xanthenes especially alpha mangostin (Yodhnu *et al.*, 2009). Mangosteen peel have been extensively used in the herbal markets (Rohman *et al.*, 2019). For the first time we report the presence of phytomelatonin in *Garcinia mangostana* peels.

MATERIALS AND METHODS

Collection and Processing of Fruit Peels

Fruit Peel of *Garcinia mangostana*, *Citrus aurantifolia*, *Punica granatum*, *Citrullus lanatus*, *Artocarpus heterophyllus*, *Citrus lemon*, *Cucumis melo*, *Selenicereus undatus*, *Musa sp.*, *Ananas comosus*, *Borassus flabellifer*, *Litchi chinensis* and *Citrullus sp.* regarded as Agro food waste were washed with the sterile distilled water and air dried. The dried peels were soaked in 100% of 99% of HPLC grade methanol in the air tight container until the colour of the methanol was changed. Cold extraction techniques were performed to extract the content of the peel. Then the extracts were allowed to dry and stored in dark, cold condition.

Quantification of Melatonin using ELISA Kit

The ELISA Melatonin kit was purchased from the Life Technologies Pvt. Ltd., About 100 microliters of the sample was placed in the 96 well Plate. Then 100 microliters of the Biotinylated Detection antibody solution were added and left to rest. The wells were washed with the wash buffer. 50 microliters of the HRP Conjugate solution was added and left undisturbed for few minutes. The reaction mixture was aspirated and washed with the wash buffer for three times. Added 90 microliters of the Substrate Reagent and kept in dark for few minutes and the reaction was stopped with the stop solution. Change of the colour from the Aqua to the Yellow indicated the presence of the Melatonin in the samples. (Wright *et al.*, 2004) Further Melatonin was quantified using an ELISA reader.

HPLC Analysis

The preliminary test and ELISA study revealed that *Garcinia mangostana* had higher melatonin concentration when compared to other fruit peels. Further confirmatory study was carried out with HPLC analysis of methanolic extract of *Garcinia mangostana* peel in Hubert Enviro Care Systems Pvt. Ltd., and the instrumentation work done on Agilent Technologies and the instrument 12060 Infinity HPLC DAD. Melatonin Standard was obtained from Life Technologies Pvt. Ltd., 10 µL of the standard and sample were injected into the column. Eclipse plus C18 Agilent column with a width of 5 mL 150 mm was used with PDA detector at 220 nm and HPLC performed with 95% of orthophosphoric acid and 5% acetonitrile as mobile phase at 100.00 mL/ min² flow rate with an elution time of 10.00 min.

RESULTS

Quantification of Melatonin using ELISA Kit

Qualitative methods showed positive coloured reaction. However further quantification was done using ELISA. ELISA may serve as best method to detect and quantify Melatonin. *Garcinia Mangostana* extract had highest melatonin of 2.2 ng/g peel when compared to other fruit peels such as *Citrus aurantifolia*, *Punica granatum*, *Citrullus lanatus*, *Artocarpus heterophyllus*, *Cucumis melo*, *Selenicereus undatus*, *Ananas comosus*, *Borassus flabellifer*, *Litchi Chinensis*, *Musa paradisiaca*, *Citrullus sp.*, *Naphelium lappaceum* had 0.2, 0.0425, 0.0078, 1.625, 0.14, 0.1092, 0.037, 0.093, 0.0078, 0.034, 0.475 ng/g peel respectively (Figure 1). It is reported that most of the fruits have low levels of melatonin (Sturtz *et al.*, 2011) except strawberries and tomato. *Garcinia mangostana* peels contain a higher melatonin when compared to the previous reports such as *Vitis vinifera* (0.01 to 0.97 ng/g) (Iriti *et al.*, 2006), *Prunus avium* L. (0.01 to 0.22 ng/g) (Gonzalez-Gomez *et al.*, 2009) *Citrus reticulata* (0.15 ng/g), *Mangifera indica* (0.70 ng/g), *Carica papaya* L. (0.24 ng/g) and *Fructus Mori* (1.41 ng/g) (Johns *et al.*, 2013).

HPLC Analysis of *Garcinia mangostana* fruit peel

Methanolic extract of the Peel was compared with the Standard Melatonin. The retention time of standard melatonin was 1.374 min (Figure 2) and the extract was 1.315 min (Figure 3). The retention time of both the standard and sample was more or less similar and the peak obtained from 1.310 to 1.409 min. Earlier Chen *et al.*, 2003 reported the detection of melatonin using HPLC-FD with 80% methanol water as mobile phase (Chen *et al.*, 2003). Standard melatonin had a retention time of 28 min. The retention time may vary according to column, mobile phase and flow rate. Some studies revealed that detection of Melatonin in HPLC is better than the ELISA technique and it was demonstrated by comparing 8 types of wines (Cheng *et al.*, 2021). HPLC for detection of melatonin is feasible and accurate compared to other methods (Reiter *et al.*, 2007).

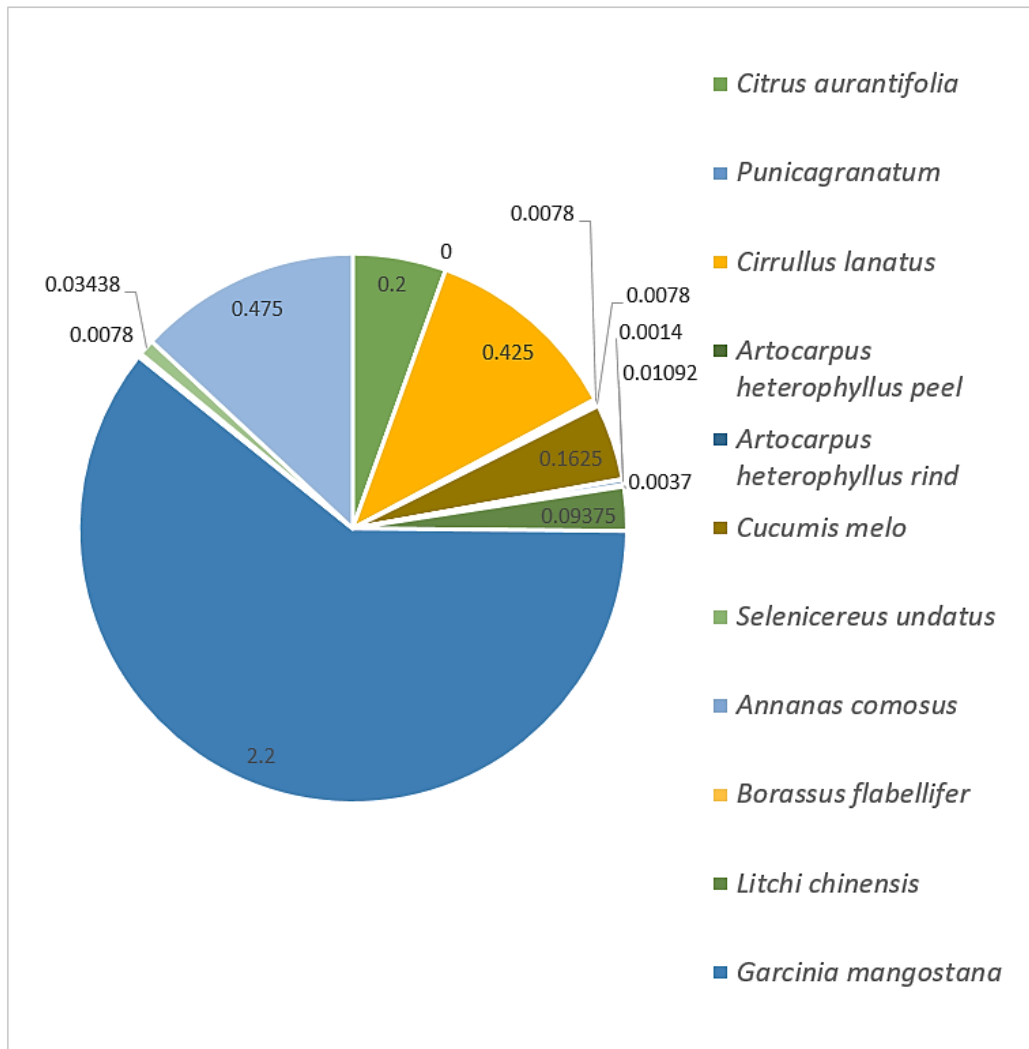


Figure 1: Presence of Melatonin in Fruit Peels.

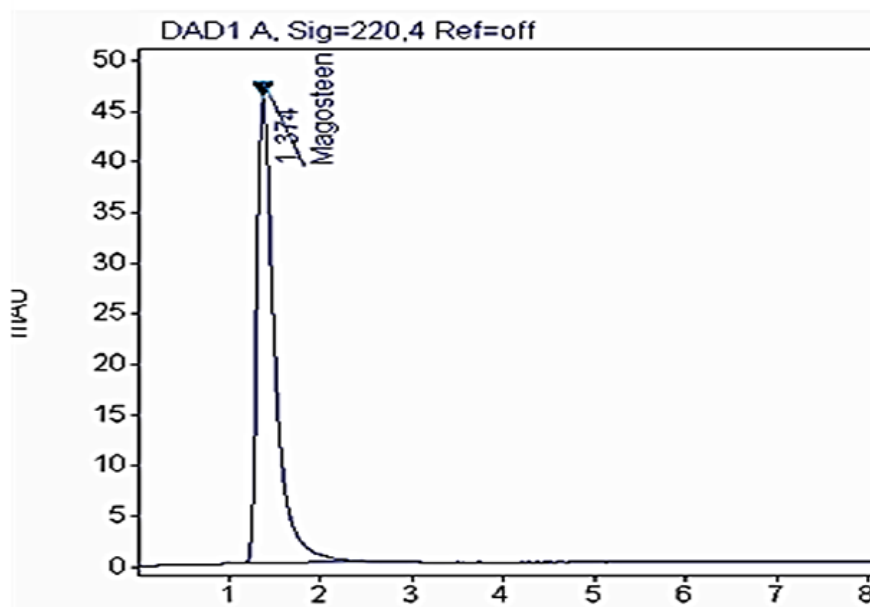


Figure 2: HPLC Analysis of Standard Melatonin. The retention time of standard Melatonin was 1.374 min.

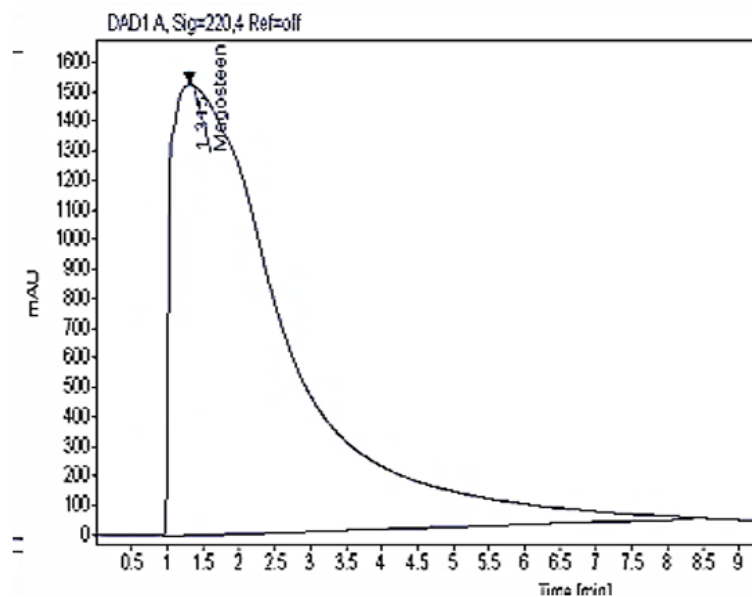


Figure 3: HPLC Analysis of Mangosteen fruit peel extract. The MeoH extract has a retention time of 1.315 min.

DISCUSSION

Phytomelatonin has been detected in large quantity in the rice varieties (Peanparkdee and Iwamoto 2019) than other plant samples (Mannino *et al.*, 2021). *Coffea canephora*, *coffea arabica*, *Mentha piperita*, *Salvia officinalis* and *Thymus vulgaris* are reported to contain the highest quantity of melatonin among the plants (Salehi *et al.*, 2019). However, it is impossible to use these plants in large scale since, they are economically and medicinally important. To maintain sustainability, screening of agro food waste is an excellent alternative approach. Peels of *Garcinia mangostana* were one of the excellent agro food waste with ample amount of useful compounds. Expensive techniques like Radioimmuno techniques and fluorescent techniques are utilized for detecting melatonin (Fraser *et al.*, 1983). For smaller samples size these techniques will be helpful but for large sample size, cost effective techniques are required. Detectable quantities of Phytomelatonin was observed in the studied fruit peels. These sources are cost effective and do not have adverse effect on human health. The phytomelatonin from these sources have multiple applications. It can be useful for human and plant health.

CONCLUSION

Melatonin being ubiquitous in function is used as dietary and sleep supplement. Synthetic melatonin has several adverse effects on human health and natural melatonin obtained plant sources are safe. The only limitation and challenge in natural melatonin is the presence of negligible or low concentration available in plant sources which are also influenced by genetical and abiotic factors. The present study reveals that Fruit peels offer a great potential source of melatonin in appreciable quantity. Among the

fruit peels investigated, *Garcinia mangostana* fruit Peel is a novel, promising source of melatonin which could be effectively used in nutraceutical and pharmaceutical industries for the production of a safer healthcare products.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Ng: Nanogram, **g:** Gram, **mL:** Microliters, **min:** Minutes, **nm:** Nanometre, **mm:** Millimeter.

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