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ESTIMATION OF SLEEP PROMOTING CHEMICALS (MELATONIN, TRYPTOPHAN AND FLAVONOIDS) IN CERTAIN NATURAL PRODUCTS

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ABSTRACT

Since Melatonin, Tryptophan and Flavonoids are both directly and indirectly responsible for generating sleep hormones, this study focuses on determination of the above compounds in assorted dietary samples which comprises of seeds of Fennel *(Foeniculum vulgare)*, peels of Orange *(Citrus recticulata)* and Banana *(Musa sapientum*) and leaves of Green Tea *(Camellia sinensis)*. Estimation was done with 1:5 ethanolic and aqueous extract of above samples in dilutions of 10%, 20%, 50%, 100% against Melatonin, Tryptophan and Gallic acid as standards. Results were recorded both– i) Spectrophotometrically and ii) using a Smartphone application PhotoMetrix Pro App at 530nm, 300nm and 510nm respectively. Comparable results showing similar trends indicated Fennel *(Foeniculum vulgare*) having highest Melatonin content whereas Tryptophan and Flavonoids are present in maximum amount in Green Tea leaves *(Camellia sinensis)* and Orange peels *(Citrus recticulata)* respectively in 100% concentration. Though Banana peel recorded considerable amount of above compounds in different concentration, Orange peel turned out to be of maximum benefit with a significant ($P<0.05$) amount of these chemicals at all concentration.

KEYWORDS

Spectrophotometer, Melatonin, Tryptophan, Flavonoids and PhotoMetrix.

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INTRODUCTION

Chemicals that regulate sleep-wake cycles and promote feelings of sleepiness and relaxation are called sleep hormones. The major sleep hormones are Dopamine, Serotonin, Melatonin which are produced by the pineal gland in response to darkness and signals the sleeping time to the body[1,2]. Although Melatonin can be consumed from diet, Serotonin and Dopamine are only formed in our body. Since Tryptophan is a precursor of Serotonin and Melatonin it can be incorporated in our body through dietary intake[3].

Melatonin (N-acetyl-5-methoxytryptamine) is a complex which consists of a cyclic compound (Indole) linked to an amino group (amine) to form an Indolamine compound. Ageing decreases production of Melatonin in brain. Hence incorporation of this compound through food becomes necessary to maintain its biological functions[4,5]. Overall, it plays a major role in reducing stress.

Tryptophan is one of the essential amino acids that must be obtained through the diet[6]. Beyond its role in protein synthesis, Tryptophan and its metabolites is responsible for sleep, circadian rhythm, and cognition.

Flavonoids that are plant metalloids, does not come under the category of sleep hormones but it gives a potential effect on sleep[7,8]. They can interact with the body's hormonal and neurotransmitter systems to potentially influence sleep patterns in regulating sleep and relaxation[9-11]. By modulating these neurotransmitter systems, Flavonoids may help promote feelings of calmness and drowsiness[12].

In search of these chemicals in naturally occurring substrates, the following samples under the category of spices, peels and leaves were selected in present study. These assorted samples are commonly used in various natural beverages which are categorised by two parts:

Alcoholic beverages (in the forms of wine/beer)

Non-alcoholic beverages (in the forms of juice/tea)[13]

Samples used for this study are as follows-

Fennel

(Foeniculum vulgare) is one of the most important aromatic seed in the world as it combines medicinal and nutritional use[14]. The main source responsible for the distinctive smell of the plant is the Fenchone and helps to reduce gastrointestinal discomfort[15,16]. Acacetin, Kaempferol are some Flavonoids present in Fennel.

Orange peels

(Citrus recticulata) as well as citrus peels in general, are excellent sources of natural bioactive compounds: essential oils, polyphenols, fibres, minerals, pectin[17,18]. In this peel, Limonene is the main component (90-95%). Also, it is rich in Flavonoids like Naringin, Hesperidin, Tangeretin which acts as an antioxidant in our body[19,20].

Banana peel

(Musa sapientum) is a waste part of Banana that are rich in various nutrients like potassium, phosphorus, calcium[21]. It shows antibacterial, antidiabetic and anti-inflammatory activity in our body[22-24]. Also, it contains saponins, alkaloids, tannins and Flavonoids like Rutin, Quercetin.

Green Tea leaves

(Camellia sinensis) can be used as a beverage for daily life. It may help to promote a good quantity and quality sleeping in presence of L-theanine. It helps to keep the stress-related hormones in check[25,26]. It contains various types of Flavonoids or Catechins like Epicatechin (EC), Gallocatechin (GC) etc.

In the present study, following estimations were executed in search of dietary supplements to promote sleep and reduce stress -

Estimation of Melatonin in ethanolic extract

Estimation of Tryptophan in ethanolic extract

Estimation of Flavonoids in aqueous extract

Initially this study was planned to be executed with aqueous solution only. Since Melatonin is sparingly soluble in aqueous buffer and is recorded to be present in negligible amount in the aqueous solutions, we determined amount of melatonin in ethanol extract of samples. Also, Tryptophan recorded a higher concentration in ethanol. Only Flavonoids concentration was recorded in aqueous extract. The transmittance of different intensities of samples were measured dually by using Spectrophotometer and a Smartphone application Photo Metrix PRO which is newly developed in Brazil to perform image acquisition and treatment of data obtained in the device itself. With this smartphone application, it is possible to prepare the calibration and determine analyte concentration of interest in the sample based on RGB system[26]. This channel has an 8-bit scale. Thus, each pixel can assume one of 28 possibilities of intensity values (0–255). All colours generated due to the mixing of these three colours can be seen in a visible spectrum range[27].

In this paper, similar to Spectrophotometer, RGB analysis is also used as an alternative method for determining concentration of various components.

MATERIAL AND METHODS

COLLECTION OF SAMPLES AND CHEMICALS

Samples

Orange and Bananas are collected from local market in Howrah. Green Tea leaves (Lipton Green Tea) and Fennel seed (JK Fennel seed) are also collected from the local markets in Howrah.

Chemicals

0.1% HCl, 2% ninhydrin reagent, methanol, ethanol, 96% H_2SO_4, MSG, 10%HCl, glacial acetic acid, sodium hypochlorite ($NaOCl\text{-}5H_2O$), $NaNO_2$ solution, NaOH are collected from Laboratories. 10% $AlCl_3$, Melatonin, Tryptophan and all chemicals were purchased from Merck Specialties Private Limited. Gallic acid and Folin reagent were purchased from LOBA CHEMIE PVT LTD.

Apparatus

SYSTRONICS Spectrophotometer 106 was used in this experiment.

Preparation of samples

The collected orange peel *(Citrus recticulata)* and Banana peel *(Musa sapientum)* are cut into small pieces. Then all the samples are weighed about 20gm and mixed in 100mL water and ethanol (1:5 ratio for each sample). They are soaked for 45 minutes with occasionally stirring. After that, basic concentration of each sample was 100cg/mL. From each 100% solution three solution of different concentration were made:

100% sample solution
50% sample solution
20% sample solution
10% sample solution

Hence, 16 samples solution were made.

EXPERIMENTAL PROCEDURE

For estimation of *melatonin*

To prepare standard solution of Melatonin, a mixture of 20mL methanol water and 10% HCl (in 70:29.9:0.1 ratio) are made and 5gm of Melatonin is added in 100 mL volumetric flask and made up the mark with ethanol. Thus, 5ml of each solution of each sample were taken in test tubes. Then 2% ninhydrin reagent (0.2g in 10mL ethanol) and 0.3mL of 96% H_2SO_4 were added. The Transmittances of the samples were measured in Spectrophotometer at 530nm[28,29] Images of all the samples are captured in PhotoMetrix PRO app.

For estimation of *tryptophan*

To make standard solution of Tryptophan, 0.025gm of Tryptophan and 25mL glacial acetic acid were taken in 25mL volumetric flask and made up with glacial acetic acid. Therefore 5mL of each sample solutions were taken in test tubes and 10% MSG, 3 drops 10% HCl, 0.2mL sodium hypochlorite were added and keep it in water bath for 5 mins. Then the transmittances were measured in Spectrophotometer at 300 nm[30]. After that the images of all the samples are clicked in PhotoMetrix PRO app.

For estimation of *flavonoids*

To make standard solution of Flavonoids, Gallic acid was taken 0.5g with 10mL ethanol in 100 mL volumetric flask and then made up with deionized water. Thus 5mL of each sample solutions were taken in test tubes. Then 2mL of $NaNO_2$, 2 drops of NaOH solution, 1-2 drops of distilled water and 0.3mL of 10% AlCl3 solution were added and keep them in dark for 15 mins[31,32], Then the transmittance of all samples were measured using Spectrophotometer at 510nm. After that the images of all the samples are clicked in PhotoMetrix PRO app.

Statistical analysis

All the experiments have been done in triplicate and data from three different experiments were subjected to analysis of variance (ANOVA) ($P<0.05$).

RESULTS AND DISCUSSION

For estimation of *melatonin*

From the experimental analyses, it is observed that the concentration of Melatonin is increasing significantly ($P<0.05$) with rise in sample concentration. Highest Melatonin concentration is recorded in Fennel seed (146.82mol/lit) and lowest in Banana peel (80.317mol/lit) in 100% concentration series of sample. In the lower

concentrations 10%, 20% and 50% concentration of Melatonin in Orange peel and Banana peel are comparable rather higher concentration is observed in Banana peel up to 20% concentration. Extent of increase in concentration of Melatonin along a concentration gradient is found to follow a pattern as: Orange peel<Banana peel<Green Tea<Fennel seed.

For estimation of tryptophan

In case of Tryptophan the concentration is also increasing considerably (P<0.05) with increase in sample concentration. Green Tea recorded a concentration of (82.626mol/lit) which is highest in the series whereas Banana peel recorded the least value (70.170mol/lit). The second highest value has to be seen in orange peel extract which exhibited almost similar concentration of Tryptophan in all concentration. As the concentration of samples increase from 10% to 100% the trend in increase of Tryptophan is as follows: Orange peel<Banana peel<Green Tea leaves<Fennel seed.

For estimation of flavonoids

Similar to Melatonin and Tryptophan, Flavonoids also showed a steady significant (P<0.05) rise in values with increase in concentration of sample. Flavonoid concentration of Orange peel tea is found to be the highest (219.027mol/lit) but Banana peel and Fennel seed also contains a considerable amount of flavonoids. Whereas Green Tea exhibited the minimum value (98.016mol/lit) in 100% concentration series.

Table No.1: For estimation of *melatonin*

S.No		In spectrophotometer				Photometrix Pro App			
	Sample	**10%**	**20%**	**50%**	**100%**	**10%**	**20%**	**50%**	**100%**
1	Fennel seed	52.753 ± 0.095	59.552 ± 0.09	63.285 ± 0.097	69.519 ± 0.09	124.914 ± 0.056	151.161 ± 0.98	160.88 ± 0.095	146.82 ± 0.114
2	Orange peel	48.045 ± 0.08	48.831 ± 0.091	51.155 ± 0.12	51.675 ± 0.99	22.243 ± 0.09	54.614 ± 0.056	82.676 ± 0.05	120.037 ± 0.095
3	Banana peel	44.551 ± 0.121	46.389 ± 0.12	53.311 ± 0.084	55.045 ± 0.088	22.534 ± 0.098	61.124 ± 0.895	70.571 ± 0.102	80.317 ± 0.088
4	Green Tea leaves	61.487 ± 0.11	70.876 ± 0.98	73.805 ± 0.12	78.41 ± 0.075	70.834 ± 0.125	84.436 ± 0.99	103.365 ± 0.112	113.015 ± 0.165

Table No.2: For estimation of tryptophan

S.No		In spectrophotometer				Photometrix Pro App			
	Sample	**10%**	**20%**	**50%**	**100%**	**10%**	**20%**	**50%**	**100%**
1	Fennel seed	57.048± 0.110	58.939 ± 0.101	64.415 ± 0.097	82.150 ± 0.111	53.516 ± 0.098	53.855 ± 0.099	55.067 ± 0.125	58.647 ±0.111
2	Orange peel	69.537± 0.098	70.170 ± 0.089	71.469 ± 0.099	76.524 ± 0.096	14.153 ± 0.065	42.838 ± 0.069	55.949 ± 0.062	65.901 ±0.071
3	Banana peel	57.871± 0.091	61.667 ± 0.099	66.299 ± 0.112	70.170 ± 0.090	8.194 ± 0.110	18.250 ± 0.101	44.431 ± 0.098	46.402 ±0.099
4	Green Tea leaves	65.748± 0.101	72.109 ± 0.133	78.996 ± 0.158	82.626 ± 0.112	57.907 ± 0.098	74.395 ± 0.089	95.561 ± 0.097	106.710 ± 0.094

Table No.3: for estimation of flavonoids

S.No		In spectrophotometer				Photometrix Pro App			
	Sample	10%	20%	50%	100%	Sample 1	Sample 2	Sample 3	Sample 4
1	Fennel seed	73.638 ± 0.096	75.798 ± 0.101	76.548 ± 0.085	77.326 ± 0.056	62.795 ± 0.0996	77.074 ± 0.096	123.596 ± 0.121	142.791 ± 0.098
2	Orange peel	83.375 ± 0.085	83.861 ± 0.075	84.347 ± 0.099	84.347 ± 0.085	156.204 ± 0.165	165.800 ± 0.11	166.065 ± 0.12	219.027 ± 0.154
3	Banana peel	76.548 ± 0.103	77.715 ± 0.098	78.520 ± 0.096	79.347 ± 0.085	68.293 ± 0.054	71.104 ± 0.0654	74.293 ± 0.0712	123.674 ± 0.078
4	Green Tea leaves	71.958 ± 0.091	79.770 ± 0.069	81.069 ± 0.074	81.069 ± 0.107	25.873 ± 0.111	35.992 ± 0.101	39.989 ± 0.106	98.016 ± 0.155

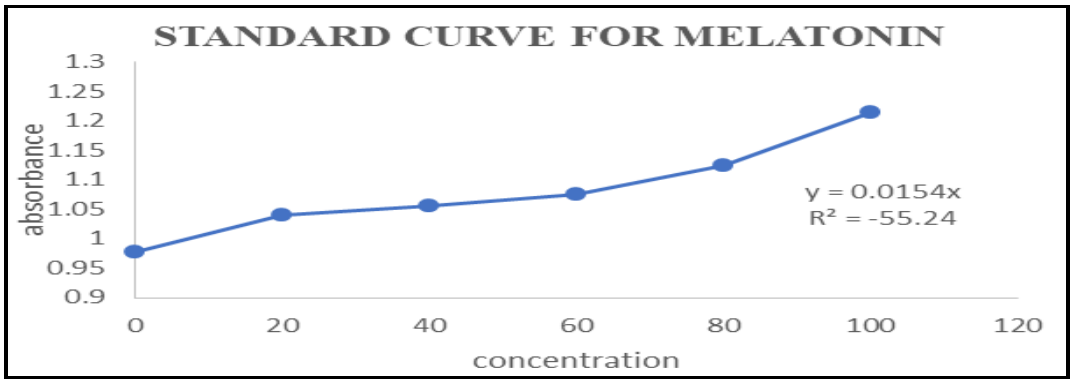

Figure No.1: Standard curve for Melatonin

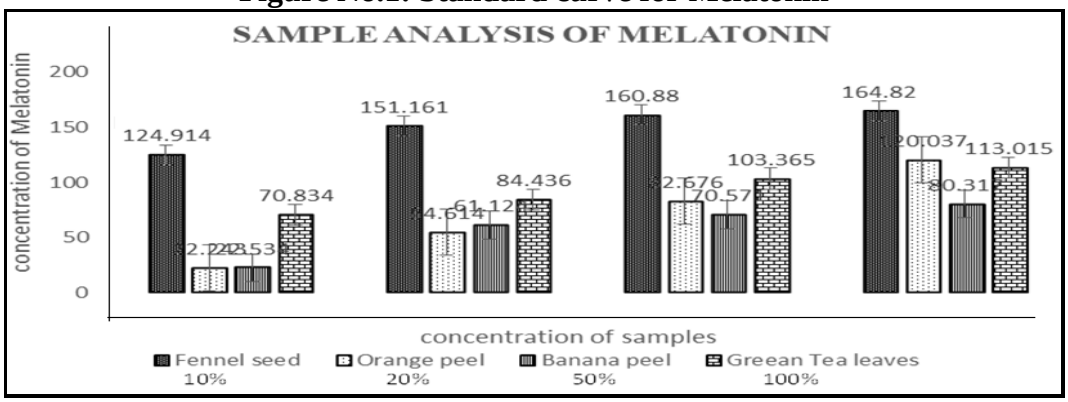

Figure No.2: Melatonin content in various samples

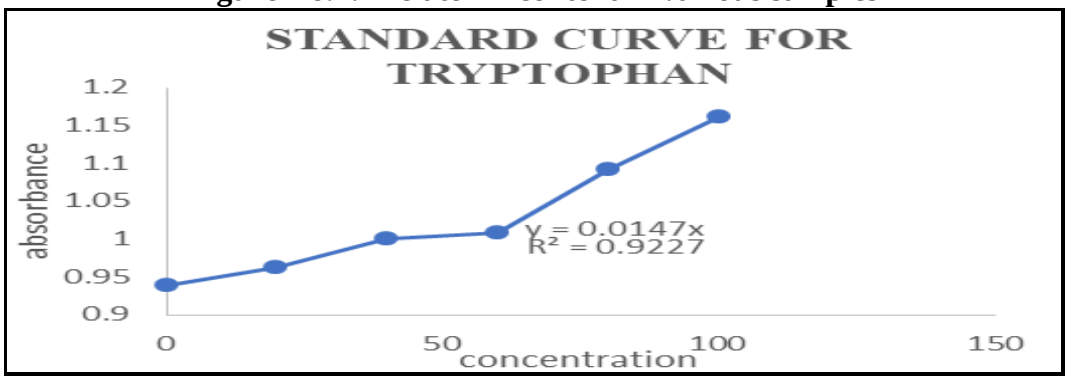

Figure No.3: Standard curve for Tryptophan

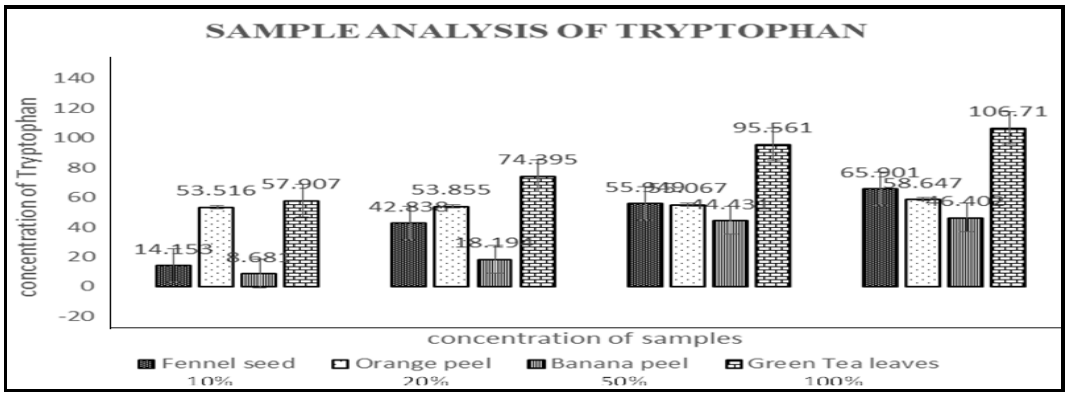

Figure No.4: Tryptophan content in various samples

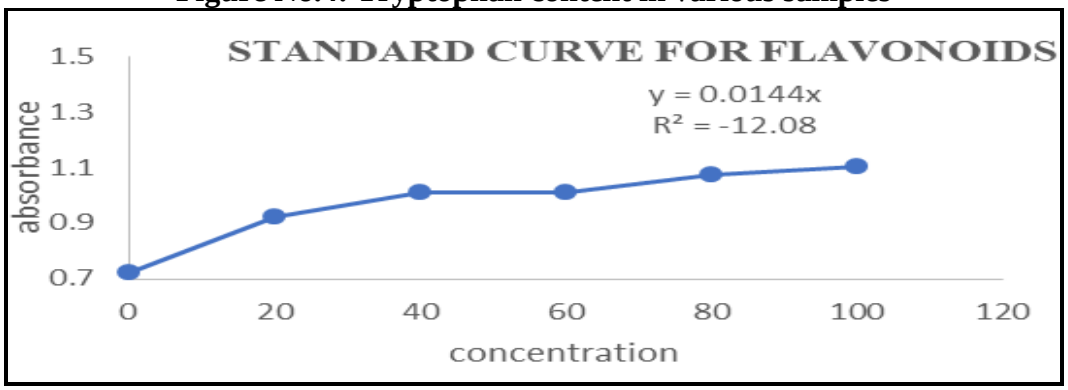

Figure No.5: Gallic Acid Standard curve

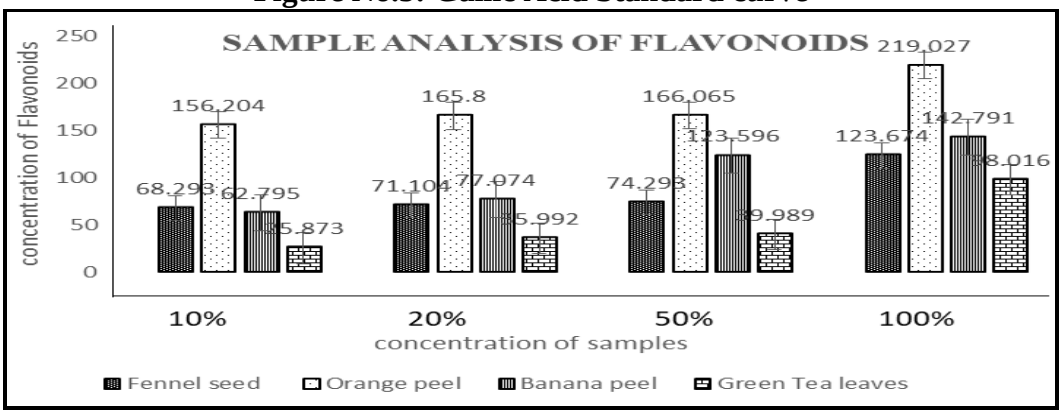

Figure No.6: Flavonoids content in various sample

CONCLUSION

From the result of this study, we can conclude that Fennel seed contains Melatonin in higher amount than others. On the other hand, Tryptophan is present in greater amount in Green Tea leaves. Hence both the component can be used in beverages and various food as they are responsible for reducing stress factors and drowsiness. It will help to increase the sleep quality. After that, aqueous extract of orange peel contains more Flavonoids which exhibit strong antioxidant activity to our body. All over graph shows us that orange peel contains all the above chemical in appreciable amount as it keeps its nutrients even in lower concentration. Since Green Tea is popular among the people, more research can help to find out dosage and effective formulation and with orange peel or Fennel seed extract to cure sleep related problems.

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CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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