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A STUDY ON PHYCOCYANIN FORTIFIED HONEY FOR ITS COMPATIBILITY AND STABILITY

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ABSTRACT

A value added product has been developed by using honey and spray dried phycocyanin is named as "Cyanhoney", it is highly enriched with anti-oxidant along with natural sweetener. The product is prepared by mixing of spray dried phycocyanin in honey with the help of planetary mixer at 30^0C±0.5, 50 RPM for 4 hrs. The product was kept in dark condition at temperature for further study. And it was analyzed for the compatibility study by using Differential Scanning Colorimetry (DSC), Fourier Transform Infrared Spectroscopy (FT-IR). The results showed that the honey and phycocyanin was found compatible with each other. Physical Appearance of Cyanhoney was dark blue in colour where as other chemical parameters remains unchanged. The Flavonoids and in-vitro antioxidant activity of Cyanhoney were performed by using $Alcl_3$ (aluminium chloride) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method respectively at interval of every 3 months for 9^{th} months and results showed that the flavonoids content (%) at 0^{th}, 3^{rd}, 6^{th} and 9^{th} months were 1.4, 1.4, 1.4 and 1.2 respectively and *In-vitro*

anti-oxidant activity (IC50 %) value at 0^{th}, 3^{rd}, 6^{th} and 9^{th} months 86 ug/ml, 173 ug/ml, 183 ug/ml and 189 ug/ml respectively. The thermal stability data was calculated by using instrument TGA Q_{50} by TA Waters Corporation and the results showed that the Cyanhoney was stable at optimum temperature. The microbial analysis of Cyanhoney were performed using serial dilution and pore plate method at 0^{th}, 3^{rd}, 6^{th} and 9^{th} month and it showed no microbial growth. Hence current study focuses new direction of value addition of honey which increases the anti-oxidant properties of honey from 841 ug/ml to 86 ug/ml. with potent antioxidant phycocyanin which increases the antioxidant properties of honey 841 ug/ml to 86 ug/ml of Cyanhoney. Hence daily intake of Cyanhoney with low cost may reduce the oxidative stress and may useful in prevention of oxidative stress related diseases.

KEYWORDS: Honey, Phycocyanin, Cyanhoney, Flavonoids, Anti-oxidant, Stability.

INTRODUCTION

At present approximately 1 lakh MT per annum honey is produced in India by 8-10 thousands of micro and small honey producers.[1,2] Honey is a natural sweetener, which constitute carbohydrates about 95% - 97% of dry weight of honey, protein content of honey is roughly 0.5%, which are mainly enzymes and free amino acids. Honey has a lots of activities like antimicrobial, antiviral, antifungal, anti-inflammatory, immune modulatory and anti-oxidant activities.[3,4] At present honey is one of the last unfortified natural functional foods so it's today's need to do the value addition of this nutritious and golden nature's gift with such a substance which increases its value not only in terms of therapeutic activity but also in terms of its monitory benefits.

Keeping in view of the above in mind a value added product of honey has been made using spray dried phycocyanin which has good potent anti-oxidant activity. Phycocyanin is phycobiliprotein characterized by a deep and intense blue color having α and β subunits contain about 160 to 180 amino acid residues, to prevent the oxidative stress related diseases at very low concentration.[5]

Phycocyanin shows a wide range of pharmacological effects with potent antioxidant, anti-cancer, anti-inflammatory activity and stimulating the immune system. It plays an anti-oxidant role in inhibiting hepatic lipid peroxidation and being helpful to liver protection, scavenges free radicals from damaged nerve cells, which could avoid DNA oxidative damage from free radicals and prevents neuronal cell apoptosis. The various researches have shown

that phycocyanin plays an effective role in arresting cancer in various cancer cell types such as breast cancer, liver cancer, lungs cancer, colon cancer, leukemia and bone marrow cancer *in vitro* and *in vivo*.[6] A recent study from Japan on phycocyanin found that it is a potent immune stimulatory effect and reduces the allergic inflammation in rodents given phycocyanin. The researchers concluded that phycocyanin enhances biological defense activity against infectious diseases through sustaining functions of the mucosal immune system and reduces allergic inflammation by the suppression of antigen-specific IgE antibody.[7] Phycocyanin exerted the central neuroprotective effect in rodent's studies- an observation which strongly suggest that phycobillins (phycocyanin) can transit the blood brain barrier (Shabana Kouser Ali et.al. 2012).[8] Hence current study highlights new direction of value addition of honey with potent antioxidant phycocyanin to increase the therapeutic activity of honey as well as monitory benefits to the producers.

MATERIALS AND METHODS

Materials

Phycocyanin was procured from Wellisen Neutraceuticals Pvt Ltd and honey was procured from a Korku community of Melghat Dist. Amravati. Dimethyl sulfoxide, Pyridine, methanol, DPPH and Alcl3 were purchased from S.D Fine chemicals, Mumbai and all are laboratory grade reagents. The media such as nutrient agar, potato dextrose agar, mannitol salt agar, macconckeys highveg agar, bismuth sulfite agar used for the microbial load detection were purchased from HIMIDEA.

Method

Preparation of Cyanhoney using optimized protocol

The Cyanhoney was prepared by using 0.5 % w/w of phycocyanin and honey, particle size 100 um±5 fortified with honey by using platenery mixer (M/S Dolar engineering industrial pvt.ltd Bangalore, SS 304) with incremental dissolution at 30^0C±0.5, 50 RPM, for 4 hrs. under hygienic condition. After through mixing the product was filled in glass bottle and kept under dark condition for further study. The scale up of optimized protocol was scaled up 15 kg for 3 batches and found reproducible without any technical difficulties.

Testing parameters for Cyanhoney

1. **Compatibility study:** The compatibility analysis of spray dried phycocyanin, honey and Cyanhoney Was carried out by using Differential Scanning Calorimetry (DSC) and Fourier-Transform Infrared spectroscopy (FTIR).[9,10] DSC analysis of Honey,

phycocyanin and Cyanhoney analyzed using DSC Q20 instrument. The thermogram of each sample measured at a temperature range 30^0C to 300^0C with a speed of 10^0C per min.

2. **Physical analysis:** It includes detection of moisture, appearance, taste, solubility, pH, total ash, acid insoluble ash, water soluble ash, total sugar and reducing sugars.

Moisture: It was calculated by using MA-150 model of Sartorius instrument with 2 gm of sample in triplicate and results were depicted in Table no. 01.

p^H: It was measured with the help of p^H meter (TOSHCON Industries Pvt. Ltd) using 10% solution in drinking water and results were depicted in Table no. 01.

Ash content: Total ash was calculated by igniting the sample at 450^0C in muffle furnace (Ambassdor P.I.D temp. controller)[11] and results were depicted in Table no. 01.

Total sugar: It was calculated by using anthrone method[12] and the results were depicted in Table no. 01.

Reducing sugars: It was calculated by using dinitrosalicylate (DNS) methods.[13]

3. **Flavonoids content and In-Vitro anti-oxidant study:[14,15]** It includes flavonoids content and *In-vitro* anti-oxidant activity by aluminium chloride (Alcl3) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method. The study was carried out for 0^{th}, 3^{rd}, 6^{th} and 9^{th} months.

4. **Stability study:** It was done by using thermal and microbial analysis.[16,17]

Thermal analysis: It was carried out by using Thermogravimetric analysis (TGA-Q_{50} by TA Waters Corporation) at 0^0C-650^0C with the speed of 10^0C per minute and results were depicted in Fig. no. 04.

Microbial analysis: It was carried out by using microbial pore plate technique at the interval of 3 months upto 9^{th} months using serial dilution method.

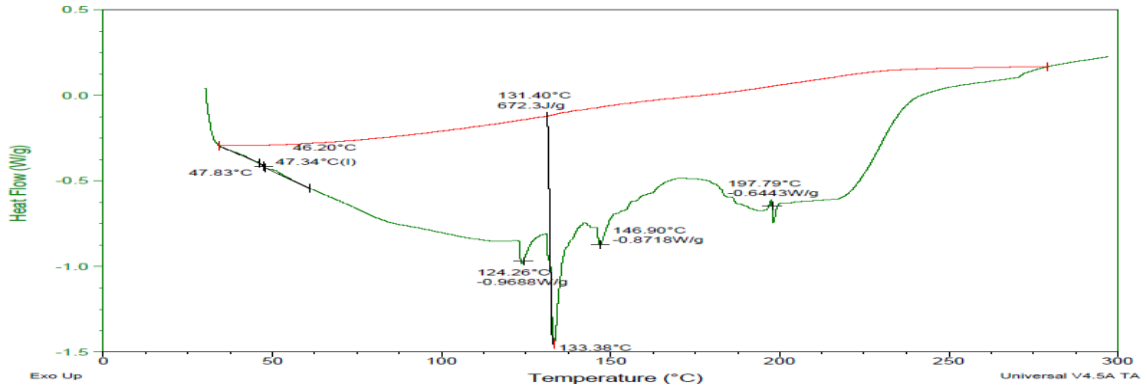
RESULTS AND DISCUSSION

1. **Compatibility study:** DSC thermogram of honey, phycocyanin and Cyanhoney were showed in Fig. no. 01. Honey produces four onsets temperature of 124.26^0C, 133.38^0C, 146.90^0C and 197.79^0C which showed there were four components in the honey. Phycocyanin produces four onsets temperature of 91.49^0C, 184.29^0C, 156.46^0C and 230.82^0C which showed there were four components in the honey. Cyanhoney produces three onsets temperature of 127.46^0C, 146.15^0C and 157.83^0C which showed there were three components in Cyanhoney. These three onsets temperature revealed that the

phycocyanin was dissolved in the honey which indicates that the phycocyanin and honey were compatible with each other and results were depicted in table no. 01.

Table no. 01: DSC thermogram results of honey phycocyanin and Cyanhoney.

Sr.no	Sample	Onset of Temp	Conclusion
1	Honey	124.26^{0}C, 133.38^{0}C, 146.90^{0}C, 197.79^{0}C	Presence of Four components
2	Phycocyanin	91.49^{0}C, 184.29^{0}C, 156.46^{0}C, 230.82^{0}C	Presence of Four components
4	Cyanhoney	127.46^{0}C, 146.15^{0}C 157.83^{0}C	Presence of three components



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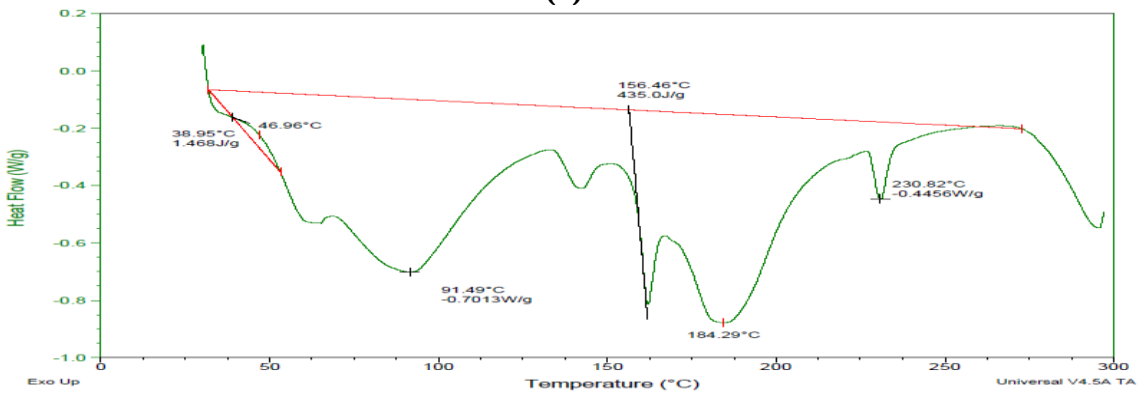

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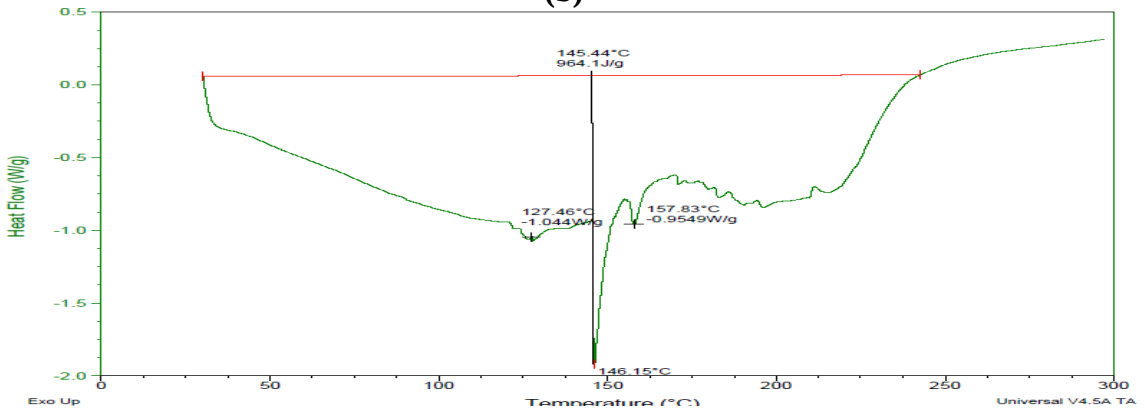

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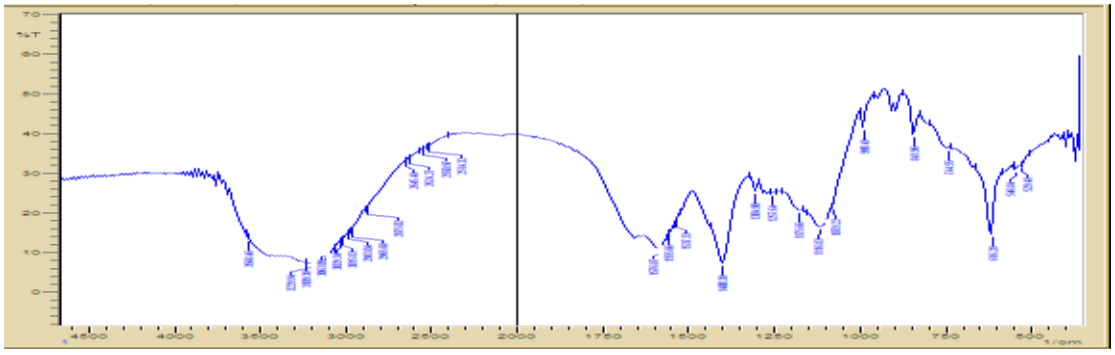
Fig. no. 01: DSC thermogram (a) Honey, (b) Phycocyanin, (c) Cyanhoney.

FTIR study of Honey, Phycocyanin and Cyanhoney were performed. The FTIR study of honey, phycocyanin showed that they are compatible with each other and from FTIR spectra of Cyanhoney at 1st, 3rd, 6th and 9th month study showed the stability and results were depicted in Fig. no. 02. The FTIR Spectra of Phycocyanin showed strong band in region between 2514 -2645 cm^{-1} which indicates it is an alcoholic group and carboxylic group, region between 990-744 cm^{-1} which indicates it is an N-H group, region between 1257-1304 cm^{-1} indicates it is an protein, region between 990-744 cm^{-1} which indicates it is an N-H group, region between 2875-2965 cm^{-1} which indicates it is an methyl group. The FTIR spectra of Honey showed the strongest intensity peak in region between 2894 -2697cm^{-1} indicates that it is an hydroxylic group, region between 866-919 cm^{-1} indicates it is an carbonyl group, region between 2950-2850 cm^{-1} indicates that it is an alkane.



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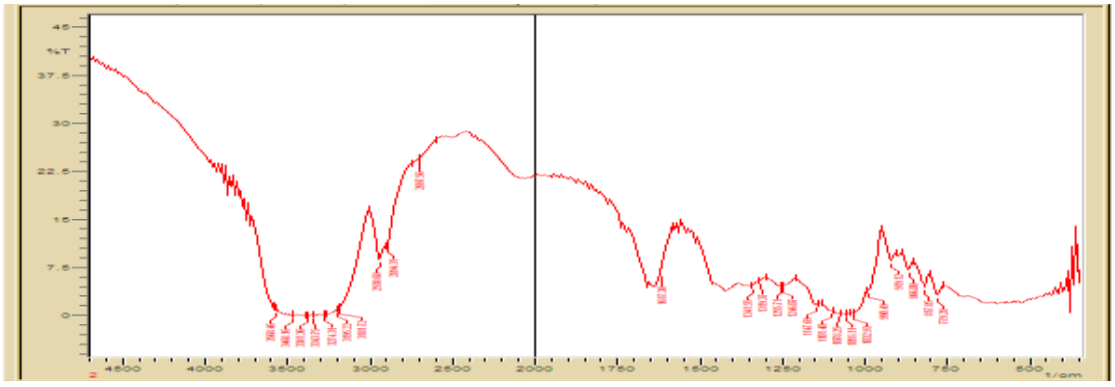

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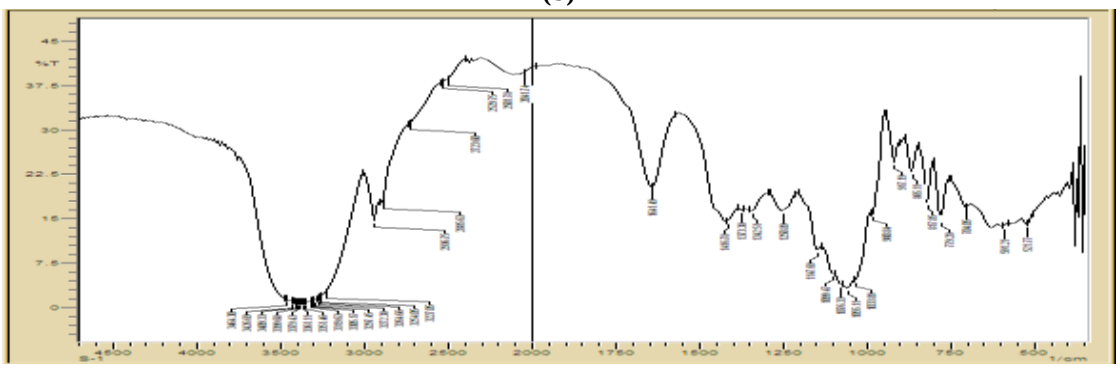

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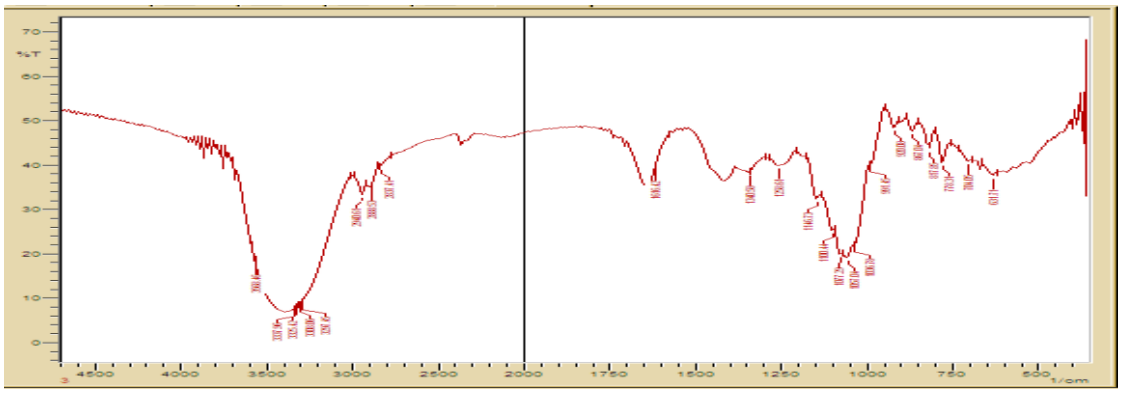

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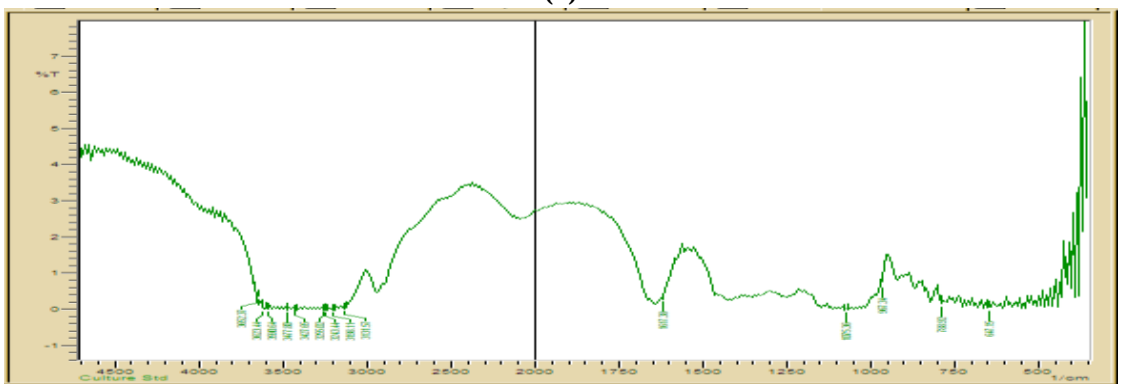

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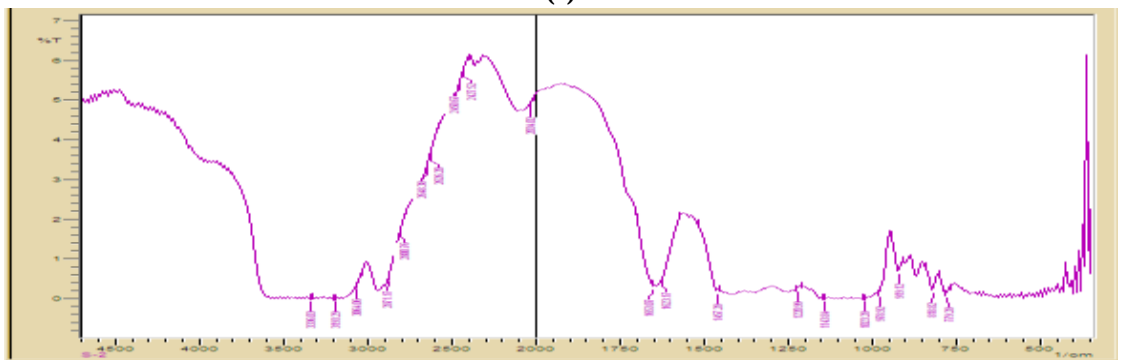

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Fig. no. 02: FTIR Spectra (a) Phycocyanin, (b) Honey, (c) Cyanhoney 1st month, (d) Cyanhoney 3rd months , (e) Cyanhoney 6th months, (f) Cyanhoney 9th months.

The FTIR spectra of Cyanhoney 0th month study showed the strongest intensity peak in 2936 - 2501 cm^{-1} indicates carboxylic O-H stretching. Also the region 3227-3464 cm^{-1} indicates carboxylic group. 1647 cm^{-1} indicates alkylene C=C, 1416 cm^{-1} indicates C-H plane bending, 1250- 1033 cm^{-1} indicates C-N, 980-917 cm^{-1} indicates acetylene, 917-704 cm $^{-1}$ 704-779 cm^{-1} indicates C-H. The FTIR spectra of Cyanhoney 3rd month study showed the strongest intensity peak in the region between 867-631 cm^{-1} indicates presence of N-H functional group, the region between 991-920 cm^{-1} indicate presence of O-H (hydroxylic) functional group, region between 2940-2888 cm^{-1} indicates the presence of methyl group, the region between 3568-3297 cm^{-1} indicate presence of carboxylic functional group. The FTIR spectra

of Cyanhoney 6th month study showed the strongest intensity peak in the region of 967-647 cm^{-1} indicates the presence of N-H functional group. 1650 cm^{-1} indicates N-H, 3477-3243 cm^{-1} indicates carboxylic functional group, 3590-3427 cm^{-1} indicates N-H stretching. The FTIR spectra of Cyanhoney 9th month study showed the strongest intensity peak 2034 -1623 cm^{-1}indicates =C-H stretching, region between 3193-3064 cm^{-1} indicates C-H stretching, region between 3336-3200 cm^{-1} indicates O-H functional group, also region between 978-919 cm^{-1} indicates O-H group, region between 900-779 cm^{-1} indicates N-H functional group.

2. Physical analysis

Table no. 03: Physical analysis parameters of optimized Cyanhoney.

Sr. no	Test	Observation
1	Appearance	Bluish in colour
2	Taste	Sweet in Taste and is palatable for all people among the group of 10
3	Moisture	12.54±1.8755
4	Solubility	Soluble in water
5	Total ash	0.3953±0.03
6	Acid insoluble ash	0.01809±0.0002
7	Water soluble ash	0.01935±0.01492
8	Reducing sugar	699.4 mg

3. In-vitro anti-oxidant study and Flavonoids content activity

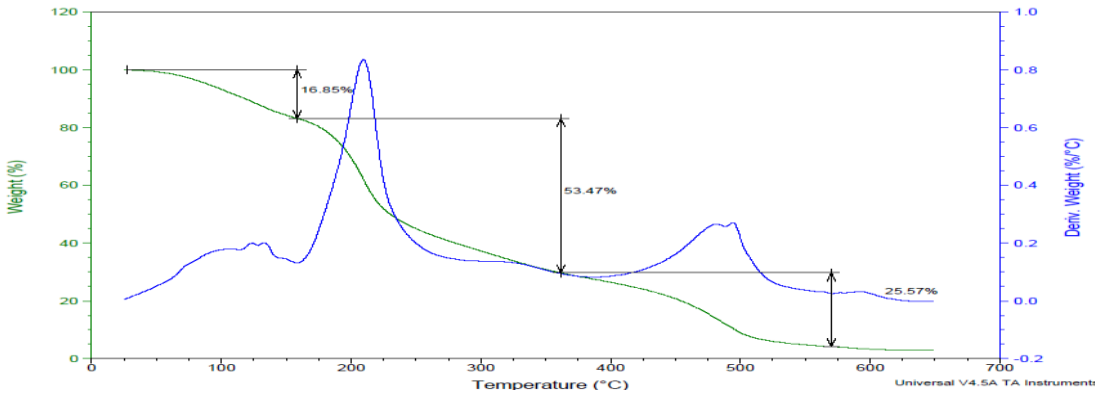
The Flavonoids content activity was determined by the Aluminium chloride method ($Alcl_3$) and the anti-oxidant activity was calculated by DPPH method. Flavonoids content and antioxidant activity were carried out up to 9th month with the interval of 3 month and Results were depicted in table no. 04.

Table no. 4: In-vitro anti-oxidant study and Flavonoids content activity.

Sr. No.	Sample	Flavonoids content (%)	Anti-oxidant study (IC50%)
1	Honey	0.9	841 ug/ml
2	Phycocyanin	1.3	67 ug/ml
3	Cyanhoney 0th month study	1.4	86 ug/ml
4	Cyanhoney 3rd month study	1.4	173 ug/ml
5	Cyanhoney 6th month study	1.4	183 ug/ml
6	Cyanhoney 9th month study	1.2	189 ug/ml

4. **Stability Analysis:** Stability analysis was performed by using thermal analysis and microbial analysis. The thermal degradation pattern of Honey, Phycocyanin and Cyanhoney has been ascertained by thermogravimetric analysis (TGA). TGA curve of

Honey, Phycocyanin and Cyanhoney were showed in Fig. no.04 (graph (a, b and c) in which the green lines mean residual loss and blue lines mean derivative thermogravimetric curve. TGA curve of honey shows that three degradation stages. The first stage is the water loss of honey and second stage is the decomposition of other components and third stage is carbonization and finally the organic matter oxidation. The TGA curve of Phycocyanin showed that four degradation stages and Cyanhoney TGA curve showed three stages of degradation and results were depicted in table no.05.



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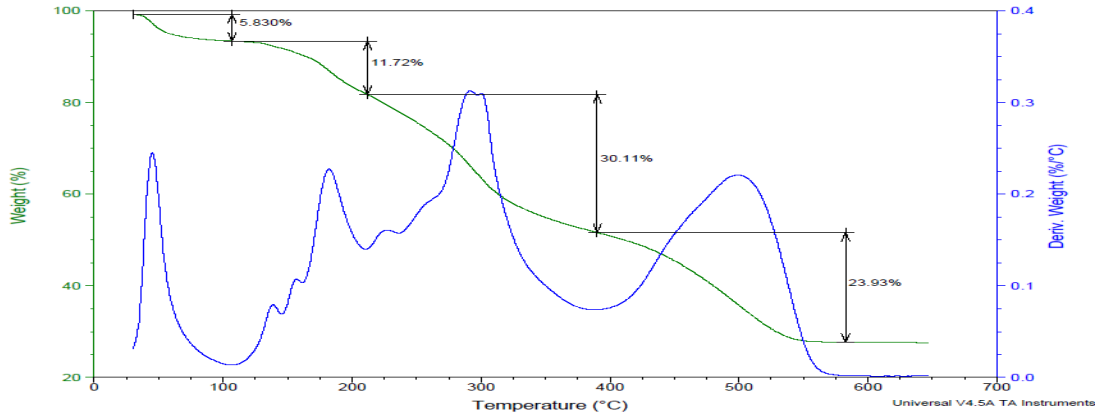

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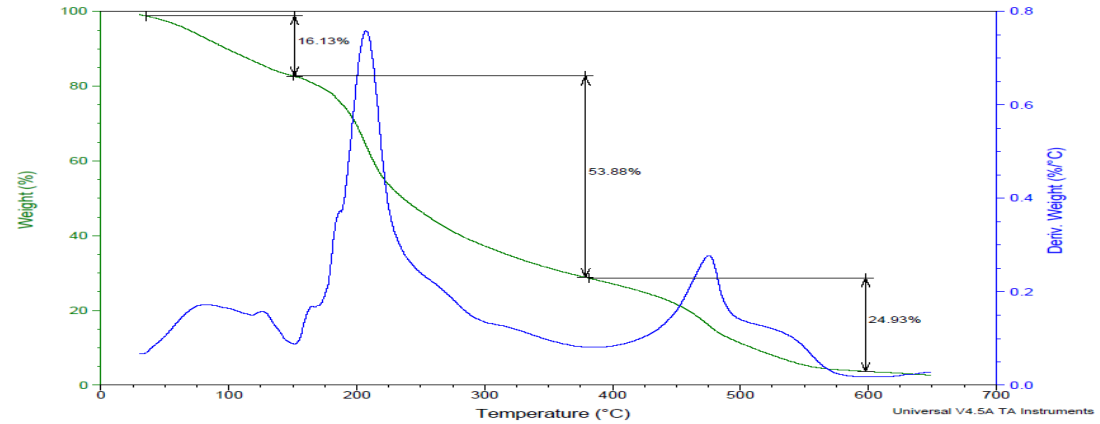

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Fig no. 05: Thermogravimetric graph (a) Honey, (b) Phycocyanin, (c) Cyanhoney.

Table no. 05: TGA thermogram results of honey, phycocyanin and Cyanhoney.

Sample	Stages of decomposition	Temp Range	Species degradation	% wt loss
Honey	1st stage	25.43^{0}C – 158.44^{0}C	Moisture	16.85
	2nd stage	158.44^{0}C – 361.58^{0}C	Other component	53.47
	3rd stage	361.58^{0}C – 569.35^{0}C	Carbonization	25.57
Phycocyanin	1st stage	40.57^{0}C – 116.12^{0}C	Moisture	5.83
	2nd stage	116.12^{0}C – 235.15^{0}C	Other component	13.83
	3rd stage	235.15^{0}C – 392.46^{0}C	Other component	27.91
	4th Stage	392.46^{0}C – 585.57^{0}C	Carbonization	24.02
Cyanhoney	1st stage	30^{0}C - 151^{0}C	Moisture	16.13
	2nd stage	151^{0}C – 382.09^{0}C	Other component	53.88
	3rd stage	382.09^{0}C – 600.46^{0}C	Carbonization	24.93

Microbial analysis: Stability study of Cyanhoney was carried out using serial dilution and pore plate method at 0th, 3rd, 6th and 9th month and was analyzed for total bacterial count, total fungal count, enterobacteriaceae, salmonella spp, s.aureus and no growth has been observed in 50 gm of sample and hence safe for consumption.

CONCLUSION

The primary observations provide that phycocyanin can be used for the value addition of honey without any compromization of existing properties of honey and phycocyanin. The results of DSC and FT-IR showed that the honey and phycocyanin was found compatible and DSC also showed the M.P of phycocyanin has been increased from 91.49^{0}C to 127.46^{0}C. *In-vitro* analysis for flavonoids and antioxidant activity showed that it was stable upto 6th month afterwards started decreasing slowly. TGA showed that the Cyanhoney was stable at optimum temperature. The microbial analysis of Cyanhoney at 0th, 3rd, 6th and 9th month showed no microbial growth. Hence current study indicates new direction regarding value addition of honey with potent antioxidant phycocyanin which increases the antioxidant properties from 841 ug/ml to 86 ug/ml to arrest the various oxidative stress related diseases.

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