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Analysis of Physico-Chemical characteristics of *Pauttika* Honey Procured from Uttar Pradesh, India

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Abstract

Background of the Study: *Ayurveda* has described eight kinds of honey with the different medicinal values that get change with the passage of time of one year. *Pauttika* honey is a specific kind of honey among the eight types of honey produced by *Pauttika* honey bee, which is indicated in the management of diabetes (*Prameha*), obesity (*Sthaulaya*), dyslipidemia, etc.

Objective: To identify and standardize the type of honey and its honey bee on scientific parameters and the characteristics as mentioned in *Ayurveda*.

Materials and Methods: Honey was procured from the natural honey hive and Standard methods were used for physico-chemical analysis, sensory evaluation, and color hunter test. Bee identification was done by an expert entomologist.

Results: Analysis of honey, showed that procured honey was *Pauttika* type of honey as described in *Ayurveda* and bee was identified as *Apis dorsata.* Researchers also found some changes in the properties of honey after the one year like in pH, HMF, Color, and Sensory evaluation.

Conclusion: The Physico-chemical analysis of honey samples confirmed the good quality of *Pauttika* honey produced by *Apis dorsata* also confirms a new variety of honey as mentioned in *Ayurveda*.

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Keywords

Ayurveda;
Apis dorsata;
Honey;
Madhu;
Pauttika Honey.

Introduction

The honey has been described as *Madhu*, *Makshika* by the *Ayurveda* scholars with different nutritional and medicinal values. Although its description is also found in different traditional systems of medicine in the world.[1–3] However, *Ayurveda* has described

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eight varieties of honey with different medicinal properties produced by eight types of honey bees. But very few scientific research studies have been done on the varieties and properties of honey as well as there are no studies on the eight varieties of honey as mentioned by the scholars of *Ayurveda*. Honey (*Madhu*) is one of the natural untreated food products produced from nectar by honey bees. It is sweet in primary taste and astringent in secondary taste; dry, cold, kindle digestion, good for color and voice, light (easily digestible), bestows softness to the body, is scarificant, good for heart and eyes, pro-constipating, enters into minute channels, reduces fat (*Meda*); indicated in diabetes, hiccup, dyspnoea, cough, diarrhea, vomiting, thirst, worms infestation and poison; alleviates all the three *Doshas*-mitigates *Kapha* by its easy digestibility *Vata* and *Pitta* by its sliminess, sweetness, and astringency. Honey aggravates *Vata*, is heavy, cold, alleviates disorders of *Rakta*, *Pitta*, and *Kapha*; is union-promoter, expectorant, rough, astringent, and sweet.[4,5] Honey, if heated or taken by a person suffering from heat becomes fatal, due to its association with poisons. It is useful in small quantities due to its properties- heavy, rough, astringent, and cold properties.[4,6] Honey has many nutrients and acts as an immunomodulator (*LehanKarma*),[7–9] gives the effect of the drug with which it combines (*Yogavahi*),[10,11] adjuvant to enhance drug absorption or bioavailability of drug (*Anupana*),[12] antibiotic,[13–15] anti-oxidant,[16] anti-fungal,[17–21] anti-inflammatory,[1] use in the management of diabetes (*Prameha*),[5] obesity (*Sthaulaya*)[5,12] and dyslipidemia,[22] etc.

Table 1: Effects of new (*Nava*) and one year old (*Purana*) honey on Body[11]

S.No.	New (*Nava*) honey	Old (*Purana*) honey
1	Nourishes to the body	Constipating
2	Does not diminish *Kapha*	Creates dryness
3	Laxative effect	Reduces fat, Scarificant greatly
4	Fresh raw honey (*Ama* honey) aggravates the three *Dosha*	Old honey (*Pakva* honey) that is preserved for many years mitigates all three *Dosha*

Ayurveda has also mentioned certain factors such as time, the vessel of storage, combination with food, exposure to heat, with which there are changes in properties of honey and its effect on the body. Just after the procurement, honey is named as new honey (*NavaMadhu*) and after the passage of one year of time is called old honey (*PuranaMadhu*). The properties and therapeutic uses of new and old honey are different (Table 1), so to get the desired effects the time of collection of honey and type of honey should be considered and advised accordingly.

Honey is produced all over the world, but the composition of honey and properties of honey is influenced by a number of factors such as geographical origin, botanical (flower) sources of nectar, environmental and climatic conditions as well as its harvesting, processing, and storage techniques.[23,24] The various varieties of honey may be grouped by the floral source; monofloral or multi-floral and bee species like *Apis dorsata, Apis melifera, Apis crena*, etc. The monofloral honey means dominating pollen grain originated from only one particular plant and multi-floral honey means no dominant pollen type in sample.[25]

Kinds of Honey

In *Ayurveda*, eight varieties of honey with specific properties have been described by different *Scholars of Ayurveda viz. Pauttika, Bhramara, Kshaudra, Makshika, Chatra, Aarghya, Auddalaka,* and *Dala.*[26] But according to *Charak Samhita*, four types of honey i.e. *Makshika, Bhramara, Kshaudra, Pauttika,* derived from specific honey bee called *Makshika, Bhramara, Kshaudra, Puttika* respectively.[5,10]

Characteristics of *Pauttika* Honey

Honey prepared by *Puttika* bees is called as *Pauttika* honey, which resembles like ghee (in color and consistency). As per the commentary

of *Chakrapani* have noted that "*pidingala Makshika mahetvah Puttikah, tudhdvam Pauttikam*" *Puttika* bees are big in size and yellowish in color[27] which lives inside hollows of big trees. This type of honey is hot in potency, causes dryness, aggravation of *Pitta*, *Rakta* and *Vata*, burning sensation, heartburn during digestion, cures diabetes,[5] etc.

Characteristics of *Apis dorsata*

Apis dorsata is a type of honey bee also called the giant honey bee, found mainly in forested areas of South and Southeast Asia. It is similar to *Apis mellifera* in appearance but is larger in size. They are about 17-20 mm in length, thus they are known as the "giant" honey bees. These show aggressive behavior against any disturbance.

The entomologist Michael S. Engel has identified the four subspecies of *Apis dorsata* found in different places of the world. The subspecies *Apis dorsata dorsata* (Indian giant honey bee) is primarily found in India. They build single large nests up to 150 cm in length and in open places on branches of high trees or buildings.

It is considered the most defensive of all of the honey bees with sharp sting up to 3 mm long and easily penetrate clothing and even the fur of a bear and are more defensive than the African honey bees.[28,29]

Various studies have reported significant differences in the physico-chemical properties, nutritional values, appearances, color and sensory parameters in new and old honey and suggest about the differences in effects after intake new or old honey.[30–36] In this manuscript, the attempt has been made to analyze and find out the differences in new and old honey properties in the *Pauttika* type of honey on standard scientific parameters.

Materials and Methods

1. Collection and storage of honey
2. Identification of honey

- Identification of bee specimens to ascertain the type of honey bee.
- Organoleptic evaluation as per descriptions in *Ayurveda* about the specific type of honey.

3. To standardize the quality of honey

- Physico-chemical analysis
- Minerals analysis
- Sensory analysis using the hedonic scale
- Color (hunter color lab) of honey
- FTIR

Chemical and Reagents

All the chemicals and reagents used were of the analytical grade and as per the standard method for the analysis of honey. The analysis and reporting of the honey samples were carried out at the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, BHU, India.

Source of Collection and Storage of Honey

The present study was carried out on honey procured from the region Uttar Pradesh, India. To ensure the authenticity of the honey sample, the collection was made directly from hives nested on the Bamboo tree, Village Karim Patti, Tanda, Distt. Ambedkar Nagar in the month of March 2018.

The procured part of the hives was collected from groves, by pressing and squeezing in the traditional method of the combs containing honey. It was filtered before storage or use at room temperature. Filtered honey was stored for one year in an airtight and light-resistant glass container for the study.

Identification of Honey and its Bee

Bee specimen Identification

At the time of procurement of honey from natural honey hives nested on a tree, few honey bees have been also collected and preserved by standard method for identification of honey bee, so that the determination of the type of honey can be done as described in *Ayurveda* and standard method.

The bees were killed using ethyl acetate after that specimens were dry mounted[37] and the identification of Specimens of honey bees was done by the expert entomologist in the Division of Germplasm Collection and Characterization, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), H.A. Farm Post, P.B. No 2491, Bellary Road, Bengaluru-560024, Karnataka, India.

Organoleptic Evaluation

The organoleptic evaluation was done two times, first at the time of procurement of fresh honey sample and second after the passing of one year.

The characteristic of honey described in *Ayurveda* was evaluated on organoleptic parameters such as color, odor, consistency, taste, etc. *In Charak Samhita,* four types of honey have been mentioned viz. *Makshika*, *Bhramara, Kshaudra*, *Pauttika*, which produced from specific honey bees called *Makshika*, *Bhramara, Kshaudra, Puttika* respectively.[5,10] Amongst them, *Pauttika* honey resembles like ghee (in color and consistency). *Puttika* bees are big in size and yellowish in color.[27]

Physico-Chemical Analysis

Ash content, acid insoluble ash, viscosity, moisture content, pH, acid value, and total soluble solids were determined as per the method described by the Association of Official Analytical Chemists (AOAC, 1990).[38] Reducing sugar content of the honey sample was determined as per the method recommended by Miller *et al.,* 1959.[39] Hydroxyl methyl furfural (HMF) content in honey was determined by following the method of Keeney and Bassette, 1959 with slight modifications,[40] the Total Phenolic Content by the method involving Folin-Ciocalteau reagent and Gallic acid standards Hinneburg *et al.,* 2006.[41] Antioxidant activity-Determination of the antioxidant potential by the DPPH inhibition method as per the procedure is given by Mimica-Dukic *et al.,* 2004, with slight modifications,[42] the vitamin C content by Plummer TD, 1978 using 2,6-di- chlorophenolindophenol with minor modifications and was expressed as mg ascorbic acid/kg honey.[43]

Table 2: Physico-chemical properties of fresh and old (one year stored at room temperature) *Pauttika* honey sample

S.N.	Parameter	Fresh honey (N=3)	Old honey (N=3)
1	Ash content (%)	0.28±0.02	0.47±0.11
2	Acid Insoluble Ash (%)	0.12±.01	0.13±.02
3	Viscosity (cP)	102.9±0.03	100±0.04
4	Moisture Content (%)	25.58±0.24	19.20±0.36
5	pH value	4.27±0.03	4.10±0.07
6	Reducing sugar (g/100g)	20.59±0.05	27.49±6.29
7	Acid value (%)	15.00 ±0.03	7.67±.01
8	TSS (°b)	68.6±0.1	75.15±0.35
9	HMF Content (mg/100gm)	4.13±0.01	11.39±0.02
10	TPC (mgGAE/g)	3±0.03	0.26±0.14
11	Antioxidant potential (%DPPH inhibition)	82.51±0.04	60.77±0.73
12	Vitamin C (mg/100g)	2±0.21	4±0.13

(TSS: total soluble solids, HMF: hydroxyl methyl furfuraldehyde, TPC: total phenolic content, N: number of replicates, Mean ± standard deviation values)

Minerals Analysis

Analysis of minerals and heavy metals was done as per AOAC, 1990 using Flame Atomic Absorption Spectrophotometer (FAAS), Model AA-7000, Shimadzu, Japan except for P, which was analyzed spectrophotometrically as per AOAC, 1990.[38]

Hedonic Rating Scale

The honey sample was analyzed for different sensory characteristics like color and appearance, body and texture, aroma and taste, and overall acceptability. Sensory evaluation was performed by a panel of 9 semi-trained judges from the Department

of Dairy Science and Food Technology, Institute of Agricultural Sciences, BHU, India. Sensory evaluation was done at 27°C and 60% relative humidity. 9-point hedonic rating scale (1 = dislike extremely, 9 = like extremely), Amerine *et al.,* 1965[44] was used for color and appearance, consistency, flavor, mouthfeel and overall acceptability.[45]

Color Measurement

L-a-b values for honey using Hunter color Lab. ColorFlex EZ model was used calibrated first using a white tile and black tile. It measures color using Three-dimensional scales, such as CIE L*a*b*, which has been developed to objectively quantify color values. This scale defines color as follows: L* (lightness) axis: black to white (0 to 100), a* (red-green) axis: positive values are red; negative values are green; 0 is neutral and b* (yellow-blue) axis: positive values are yellow; negative values are blue; 0 is neutral. All visible colors can be quantified within this 3-D rectangular space.[45]

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Infrared spectra of the honey sample was recorded using the PerkinElmer spectrum version 10.4.3 FTIR spectrophotometer. A pinch of honey has been taken and placed over the crystal present on stage. The IR spectrum was scanned between 4000 to 400 cm^{-1} and transmittance was recorded. Before scanning the sample, the background signal was also recorded. The peaks thus obtained were matched against the IR interpretation chart and the functional groups were noted.

Results and Discussion

Identification of Honey Bee and Organoleptic Evaluation

On naked eye inspection, the color of the bees was yellowish, black. The entomologist has identified the specimen's honey bees as *Apis dorsata*.

As per *Ayurveda*, the *Puttika* bees are big in size and yellowish in color,[27] which lives inside hollows of big trees. Thus, the characteristics found in the sample of honey bees, are similar to *Apis dorsata* as per the characteristics described in *Ayurveda* and the report of the entomologist. The physical characteristics of the honey samples were also the same as the characteristics of honey produced by *Puttika* honey bees as the color and consistency of honey was similar to ghee.

Fig.1: Honey bees specimen

Fig.2: Honey bee (*Apis dorsata*)

Fig.3: Fresh honey

Fig.4: Old honey

Fig.5: Size of honey bees

Thus, researchers have determined that the collected honey was *Puttika* type of honey on the basis of similarity of characteristics of honey as well as sample honey bee (Figure 1,2,3,4 & 5).

Picture of Honey Bee (*Apis dorsata*) and Honey (New and Old)

Tables (2,3,4&5) show the mean values and standard deviation of the basic characteristics of the physico-chemical analysis, AAS to obtain mineral contents, sensory evaluation, and color appearance of honey. The obtained data could be discussed as the following:

Physico-Chemical Analysis of Honey

To standardized and validate the *Pauttika* honey, physico-chemical characteristics of the freshly tested *Pauttika* honey is compared with one-year-old *Pauttika* honey (Table 2) along with previous studies. Codex Alimentarius Commission (CAC, 2001) was used as a standard for comparison.

Ash Content and Acid Insoluble Ash

The obtained ash values were 0.28% in fresh honey and 0.47% in old honey, which indicates the cleanness of the product. However, since these values were less than the limits allowed by CAC for floral honey (≤0.6%). Samat *et al.,* 2017 and Islam *et al.,* 2017 reported the ash content in fresh honey 0.14± 0.01%46 and 0.14±0.0%,[47] respectively. Another study on big honey has shown almost similar ash content i.e. 0.41±0.037%[48] as the one-year-old honey value in this study. The obtained acid insoluble ash values were 0.12% and 0.13% in fresh and old honey, respectively.

Viscosity

The value of viscosity was 102.9 (cP) (at 20 rpm) 37°C in freshly procured *Pauttika* honey and 100 (cP) in old honey (Table 2), while other studies have reported 0.76 ± 0.22 (Pa•s) in Tualang honey and 0.53 ± 0.06 (Pa•s) Gelam honey.[49] Viscosity is one of the most significant physical and sensory characteristics of honey, that affects the quality and influenced by temperature, moisture content, as well as the presence of crystals and colloids in the product.[50]

Moisture

The obtained moisture content was 25.58% in the fresh honey and 19.20% in the one-year-old honey (Table 2). As per the international regulations CAC (2001) the acceptable, good quality of the honey[51,52] contains the maximum value of moisture is ≤21%. But a study of S.P. Kek *et al.,* 2018 have shown higher values of moisture content; 26.62±0.50g/100g in Tualang and 27.41±0.46g/100g in Gelam honey procured by *Apis dorsata* bees.[49] The cause of the higher moisture content may be unprocessed honey, collection at the time of rainy season,[53] high moisture leads to undesirable fermentation of the honey during storage caused by the action of osmotolerant yeasts, which results in the formation of C_2H_5OH (ethyl alcohol) and CO_2 (carbon dioxide). The alcohol can be further oxidized to acetic acid and water, which leads to a sour taste.[54,55] The lower moisture content observed in the rest of the samples may be attributed to the hotter weather and the decreased rainfall, especially from the beginning of the year to the middle of the year and as it contributes to its ability to resist the growth of micro-organisms, fermentation, and granulation during storage thus promoting a longer shelf life.[36,47,56–58]

pH Value

The parameter of pH is important during honey extraction and storage because pH influences its texture, stability, and shelf life.[49,59] Honey is characteristically acidic in nature, it consists of organic acids, particularly gluconic, pyruvic, malic and citric acids; lactones; esters; and some

inorganic ions, such as phosphate and chloride.[60–62] The obtained values of pH are 4.27 in the fresh honey and 4.10 in the old honey (Table 2), which were within the recommended limits (pH 3.42 to 6.10) for fresh honey.[63] It indicates whether the various processing conditions or 1 year of storage does not significantly affect the pH of honey. pH value is a useful criterion of possible microbial growth. The high acidity of honey is an indication of the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey i.e. flavor and stability against microbial spoilage.[55,64–66] The variation in pH of different honey samples are described to be due to the floristic composition and floral diversity of the regions.[67,68]

Acid Value and Total Soluble Solids (TSS)

The acid values were 15.00mgKoh/g and 7.62mgKoh/g and Total soluble solids 68.6°b and 75.15°b in freshly procured and old honey (Table 2), respectively.

Reducing Sugar

Sugars are the primary constituents of honey, and the key sugars are fructose and glucose, which is reducing or invert sugar. The total reducing sugar contents in the *Pauttika* honey samples were 20.59g/100g in the fresh honey and 27.49g/100g in old honey (Table 2), which was less than the limit ≥45 set by the CAC.[51] The room temperature storage may increase the reducing sugar contents of honey because higher temperatures result in the evaporation of water, thus resulting in more concentrated honey.[61]

Hydroxyl Methyl Furfuraldehyde (HMF) Content

HMF is used to determine the degree of deterioration of the honey and to indicate the purity and freshness of honey. The mean HMF value was 4.13mg/100g and 11.39mg/100g in fresh and old honey respectively (Table 2), which were very less than 60mg/kg suggested by CAC.[51] M Rabiul Islam *et al.,* 2017 have reported a similar finding of HMF (4.4±0.2 mg/100g) in fresh honey.[47] HMF is usually present in traces in fresh honey, but its levels tend to increase during processing and/or due to aging and also influenced by several other factors, such as pH, temperature, duration of the heat process, storage conditions and floral source; therefore, HMF levels provide an indication of overheating and poor storage conditions.[66,69]

Total Phenolic Content

Total phenolic content was 3mgGAE/g and 0.26mgGAE/g in the fresh and old honey respectively (Table 2), the finding of old honey was something similar to 0.8 mg/g of a study of Abdulaziz S. Alqarni *et al.,* 2012.[70] Total phenolic content is a good criterion to determine the quality and curative properties of honey.[71] Reportedly, polyphenols, flavonoids, and anthocyanins are the major bioactive compounds in foods and beverages that contribute significantly to the taste, texture, color, appearance, antioxidant activity, and functional properties.[72] It may be beneficial for health and may give the therapeutic impact of chronic diseases.[46,73] The presence of phenolic acids and flavonoids in honey samples might act as a proton donator leading to the formation of acidified honey with low pH value.[74]

Antioxidant Activity (%DPPH Inhibition)

DPPH is a stable nitrogen-centered radical and has been widely used to test the free radical scavenging ability of various samples.[67] The percentage of DPPH scavenging activity of *Pauttika* honey was 82.51% and 60.77% in the fresh and old honey respectively (Table 2), which were similar to the previous studies; Bangladeshi honey 70.1±0.9mg/ml[47] and stored more than one-year results was 62.77± 24.18%.[58] This may be due to the presence of high amounts of polyphenols as well as other functional components in these honey types.[47]

Vitamin C

Ascorbic acid is one of the non-enzymatic antioxidant substances present in honey.[64,75,76] The ascorbic acid content of the fresh *Pauttika* honey was around 2mg/100g and 4mg/100g in old honey (Table 2), while a study by M Rabiul Islam *et al.,* 2017 has analyzed the vitamin C content in 10 samples of honey (*Apis dorsata*), the mean value obtained was 107.3±10.8mg/kg.[47] The high content of ascorbic acid indicates a high antioxidant capacity of honey.[77]

Minerals Content

Analysis of mineral content is an important index of possible environmental pollution, the presence

of toxic content, and a potential indicator of the geographical origin of honey. Mineral contents in the honey of the *Pauttika* honey are shown in Table 3. In this study, a total of four elements i.e. Ca, Fe, Mg, and Zn were quantified, and the obtained values of these minerals are Ca (2.93ppm), Mg (4.47ppm), Fe (0.34ppm), and Zn (4.47ppm). It is well known that mineral elements are involved in various physiological and metabolic processes, especially in bone formation, blood clotting, muscle contraction, and enzyme activity. Therefore, honey is popularly used as a good source of nutritional supplements.[47]

Table 3: Minerals analysis of fresh *Pauttika* honey sample

S.N.	Element	Fresh honey (ppm) (N=3)
1	Ca	2.93±0.01
2	Mg	4.47±0.17
3	Fe	0.34±0.01
4	Zn	4.47±0.17

N: number of replicates

Table 4: Sensory evaluation score (by 9 points hedonic rating scale) of fresh and old (one year stored at room temperature) *Pauttika* honey (Mean ± standard deviation values)

S.N.	Sensory evaluation	Fresh honey	Old honey
1	Color and appearance	7.0±0.00	7.2±0.60
2	Consistency	7.0±0.00	7.0±0.5
3	Flavor	7.34±0.5	7.7±0.67
4	Mouthfeel	7.0±0.00	7.8±0.67
5	Overall acceptance	7.08±0.12	7.4±0.33

Sensory Evaluation of Honey

The data pertaining to sensory evaluation of honey fresh and old honey stored at room temperature (Table 4) showed that statistically higher scores (6.9) were given to honey at the fresh stage and the scores for sensory quality were increased during storage. The overall acceptance score in old honey was 7.4 which suggests the more acceptability of old honey than fresh.

Table 5: Color (hunter color lab) test of fresh and old (one year stored at room temperature) *Pauttika* honey sample

Observation	Fresh honey (N=3)	Old honey (N=3)
L Scale	35.33±0.14	12.99±0.01
a Scale	1.78±0.035	9.96±0.04
b Scale	13.42±0.19	15.08±0.14

The color and appearance of fresh honey were liked moderately (score 7) which was less than the one-year-old *Pauttika* honey (liked very much score 7.2). The score of Consistency, flavor, and mouthfeel were 7, 7.34, 7 in old and 7, 7.7, 7.8 in fresh honey, respectively. The higher sensory evaluation scores in old honey than fresh honey suggest that the acceptability of old honey was more than fresh honey. So the overall acceptance of one-year-old honey was more acceptable than the fresh honey on the hedonic rating scale.

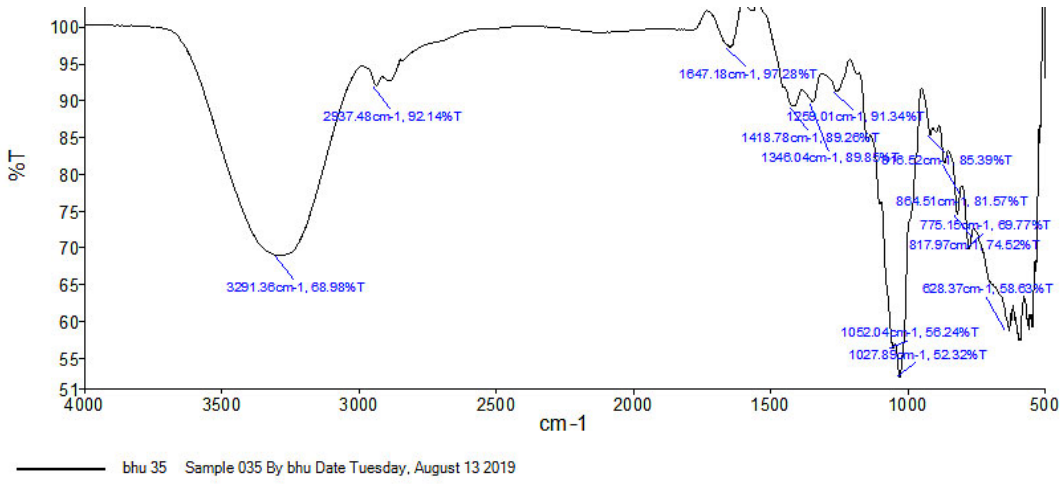
Color (Hunter Color Lab)

A different trend was observed in the color hunter lab of honey after subjection to during the storage period, with respect to botanical origin (Table 5). In particular, L* values were decreased in *Pauttika* honey old sample after the passing of one year. Red components values (a*) and yellow components values (b*) were increased in *Pauttika* honey old sample after the passing of one year.

The analysis of the hunter color lab of *Pauttika* honey at the fresh and old honey whereas L* which shows lightness and darkness of the color whereas a* scale shows redness and greenness of the product and b* scale shows yellowness and blueness of the product. So, the values of L* scale in fresh honey showed darkness, which was less dark in comparison with old honey. The value of a* scale and b* scale of fresh honey were having less redness and yellowness in comparison of old honey respectively. Overall the color of the fresh honey sample was grayish, yellowish with less quantity of redness whereas in the old honey was darker with yellowish and redness.

Table 6: FTIR spectroscopy of old (one year stored at room temperature) *Pauttika* honey

Peak no	X(cm^{-1})	Y(%T)	Group	Compound class
1	3291.36	68.98	O-H Stretching	Alcohol/Amine/Carboxylic acid
2	2937.48	92.14	C-H Stretching	Alkane/aldehyde
3	1647.18	97.28	C=0 Stretching	Primary amide/conjugated acid or aldehyde/tert. or secondary amide/lactam/unsaturated ketone
			C=N Stretching	Imine or oxime
4	1418.78	89.26	O-H Bending	Alcohol
			S-H Stretching	ThioPhenol
5	1346.04	89.85	S=O Stretching	Sulfonate/sulfonamide/sulfuric acid
			C-O Stretching	Aromatic ester
6	1259.01	91.34	C-F Stretching	Fluoro compound
			C-O Stretching	Alkyl aryl ether
			S=O Stretching	Primary alcohol
7	1052.04	56.24	C-F Stretching	Sulfoxide
			C-N Stretching	Amine
8	1027.89	52.32	CO-O-CO Stretching	Anhydride
			C-H Bending	1,2,4- trisubstituted
			S=O stretching	Sulphoxide
9	916.52	85.39	C-H Bending	1,3 disubstituted
10	864.51	81.57	C-H Bending	Alkene
11	817.97	74.52	C=C bending	Alkene
			C-H Bending	1,2,3 trisubstituted
12	775.15	69.77	C=C bending	Olefin
			C-H bending	Alkane
13	620-515	Strong	C-X stretching	(X = Cl, Br, I) Halo compound



Source Spectra Results	
Spectrum Name	**Number Of Peaks**
bhu 35	20

Fig.6: FTIR Spectrum of *Pauttika* honey

FTIR (Fourier-Transform Infrared Spectroscopy)
FTIR Spectrum is a technique to measure the wavelength of a sample and find out the peak value, characteristic bands of different functional groups. The value obtained in the *Pauttika* honey sample is mentioned in Table 6 & Fig 6.

FTIR spectroscopy has shown the presence of different characteristic peak value with various functional compounds such as alcohol, carboxylic acid, alkyne, amine salt, alkane, imine/oxime, alkene, conjugated alkene, cyclic alkene, alkene, sulfate, phenol, sulfonate, sulfonamide, sulfuric acid, sulfone, fluoro compound, aromatic ester, alkyl aryl ether, primary alcohol, sulfoxide, amine, 1,2,4- trisubstituted, 1,3 disubstituted, halo compound, 1,4 disubstituted or 1,2,3,4- tetrasubstituted, 1,2,3 trisubstituted, 1,2 disubstituted that may be responsible for various medicinal properties.

Strength, Recommendation, and Limitations of the Study
The naturally procured honey has been identified as *Pauttika* honey a specific kind described in *Ayurveda* is one of the strengths of the present study. *Pauttika* honey has various medicinal properties; *viz.* hypolipidemic action, indicated in diabetes, etc. which can be further useful for the treatment of some kind of diseases. Characteristics of honey are influenced by a number of factors such as geographical origin, botanical (flower) sources of nectar, environmental and climatic conditions as well as it's harvesting, processing, and storage techniques, but researchers have taken only one sample of honey from one state. So, there can be further research on various samples of honey and honey bees procured from the different places in India, and identification of eight kinds of honey bees and honey could be done as mention in *Ayurveda* texts. The experimental and clinical studies could be conducted on various kind of honey and their properties as mentioned in the Ayurvedic text.

FTIR spectroscopy has reported various functional groups. Further research on the analysis of sub-compounds of this honey may be conducted.

Conclusion
This study was the first attempt to standardize and validate *Pauttika* honey as mentioned in *Ayurveda* texts and as per modern parameters viz. physico-chemical analysis, organoleptic characters, hedonic scale, and hunter color lab, etc. The honey bee was identified as *Apis dorsata* and the Physico-chemical analysis of honey samples confirmed the good quality of honey according to the standards set by the Codex Alimentarius Commission. FTIR spectroscopy showed the presence of various functional compounds that may be responsible for

various medicinal properties. The standard analysis of honey sample confirms that the procured honey was *Pauttika* types of honey among the eight kinds of honey as already mentioned in the ancient texts of *Ayurveda*. Analysis of fresh and old honey showed a difference in the organoleptic evaluation, hedonic scale, color, and physico-chemical analysis which is consistent with the difference in properties of fresh and old honey as mentioned in *Ayurveda*. The overall acceptability of old honey was found better than fresh honey after getting the above analysis.

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Conflict of Interest

The authors have no conflict of interest to declare.

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