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A Comparative Study of Different Monofloral and Multifloral Honey Samples from Northern India for their Health Promoting Activities and Physicochemical Parameters

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Abstract: *Since ancient times honey is considered good for health and has been used in almost all civilizations of world. The physicochemical characteristics of honey are mainly affected by bee forage, geographical and climatic conditions and thus are responsible for the active phytoconstituents present in them responsible for its health promoting activities. In this study one branded honey (Patanjalihoney) and three locally available honey (Eucalyptus, Litchi and multifloral honey from northern India) were examined for their physicochemical parameters and their health promoting activity (antioxidant and antibacterial activity).All the physicochemical parameters of the samples were within the range as provided by FSSAI (Food Safety and Standards Authority of India). The antioxidant activity of branded honey was found to be greater than any other sample. However, the total phenolic content was lesser than that of monofloral honeys. The total flavonoid content was more than monofloral honeys but less than that of other non-branded multifloral honey. The diluted sample of all honey analyzed did not exhibited inhibitory effect against Pseudomonas aeruginosa, although the zone of inhibitions were obtained from undiluted honey samples against Bacillus cerus, Staphylococcusaureus, Eschericiha coli, Salmonellaspp, Shigellaspp. The study is the need of hour to reveal the health promoting activities and physicochemical parameters which are responsible for quality of honey.*

Keywords: *physicochemical characteristics, antioxidant activity, antibacterial activity, functional food, life style diseases.*

1. INTRODUCTION

In India as well as in other countries honey has been used since ancient times for its health promoting activities. Nowadays it is considered to be as one of the important functional food and is greatly consumed in almost all Indian households. Besides functional food, it is considered as good sweetener, antiseptic, pre and probiotics, and also have shown immunmodulatory, antitumor, anticancer, antimicrobial, antibacterial, anti-inflammatory and antioxidant activities in many researchers conducted so far [1,2].

The quality parameters of honey are governed by the regulatory bodies of the countries itself however an international platform is also there to present the guidelines for international trading of honey. The regulation set by the Alimentarius strictly instruct that the consumers have the right to receive truthful information about the food they are going to consume. The honey should not have any added ingredients , any foreign material, aroma, tainted absorbed substances during processing or storage[3]. However the various physicochemical parameters of honey varies slightly accordingly with the bee forage, geographical locations, climatic conditions etc and thus to assess the quality parameters with authenticity is chiefly required [4]. The medicinal attributes are mainly affected by its chemical composition, and physical appearance is affected with methods of extraction, processing, packaging and preservation techniques [5]. In numerous researches the nutritional assessment, identification of various biochemical compounds, quality parameters have been studied, but less work is done on its health promoting activities. Honey is regarded as good

antioxidant source mainly due to various chemical compounds present naturally in it. The various class of compounds includes vitamins, polyphenols, flavonoids, various enzymes, few minerals as iron and copperetc [6,7]. In recent investigations honey has been proven to be effective in many therapeutic and biological activities like anti-inflammatory, antimicrobial, antioxidant etc and confirms its biological importance for the treatment of wounds, skin diseases, gastrointestinal disorders and many others [8]. The physicochemical comparison along with its health promoting activities between monofloral, multifloral, branded honey sample has been not explored in much detail in India probably because of diverse geographical locations, climatic conditions, bee forage and other processing and storage conditions. Thus the major area of interest is to find the variation among monofloral and multifloral (branded and non-branded) honey with respect to antioxidant activity, antibacterial activity, and physicochemical parameters so that assessment of their health promoting activities can be highlighted after investigations and thus meets a void in research.

2. Materials and Method

All the samples were procured in sufficient amount and the study was performed with same sample batch and were without any preservative. The monofloral litchi and eucalyptus honey as well as seasonal non branded honey were procured from the local beekeepers and within same duration Patanjali honey (branded multifloralhoney) was also purchased so as to maintain uniformity. All the methods used were in accordance with the national and international standards routinely followed in Honey industries.

2.1. Preparation of Honey Samples

All honey samples were prepared according to the guidelines provided by IS Standard, Annexure J, Clause6.1 and were free from suspended solids, granulation and any form of crystallization.

2.2. Evaluation of Physicochemical Parameters

The physicochemical parameters were determined according to the methods described in 'Indian Honey Specification' by the –FSSAI 2020 as well as other standard methods followed in honey industries globally. All the samples were taken in triplicate. The honey samples were diluted in 10% distilled water and then pH was determined using (HI 9025-HANNA) pH instrument. The electrical conductivity was calculated as described by World Network of Honey Science (range 0.1 - 3mS.cm^{-1} and results expressed in milli Siemens per centimetre mS/cm).The moisture in honey samples was detected by refractometer method as per standard provided by International Honey Commission 2009. The colour of samples were determined by using HANNA instrument (In House Method, used in Honey Industries).10 g of each sample was slightly warmed and let stand to clear bubbles as far as possible, then poured very carefully into 44mm cell to avoid entrapped air and the cuvette was covered with a cap and readings were taken and then matched with the table given by USDA classification for honey samples and the related mm P fund values. The water insoluble content was determined using method prescribed by FSSAI where 20 gram of each honey was dissolved in about 200ml of water at about 80 ^{0} C , mixed well and further dried in a crucible in the oven and kept to obtain ambient temperature in a desiccator containing an efficient desiccant such as silica gel. The sample was weighed, filtered, washed extensively with warm water until free from sugars. The crucible was dried at 135 ^{0} C for an hour, cool in the desiccator and weighed once attain a constant weight. The results were calculated as percent insoluble matter in 100 grams of sample. Similarly acidity in terms of formic acid ,total ash content, total reducing sugars, sucrose content proline, diastase enzyme activity, fructose glucose ratio and hydroxyl methyl furfural (HMF) were calculated using standard protocols provided by FSSAI (04B-007;006;004; 005;013; 010; 005and 009 -2023 respectively).

2.2.1. Total Polyphenols and Total Flavonoids

The total polyphenols in honey samples were determined by the method described by Singleton et al. [9] with some modification. The total polyphenols in all honey samples were determined by UV spectrophotometer. 1.0 gm of honey samples were dissolved in 10 mL of distilled water then from it 1.0 mL of sample was taken out in test tube and to it 1mL Folin Ciocalteu reagent was added and the tubes were incubated for 5 minutes. Then 5m L of 10 % sodium carbonate solution was added in it and incubated in dark for 1 hour. The absorbance was recorded at 760 m using UV spectrophotometer. The same procedure was followed for gallic acid to plot linearity. The

total phenolic content was reported as mean value of triplicate assays and expressed as milligram of gallic acid equivalent (GAE) in gram of honey. Similarly for determining total flavonoid content in honey samples 10 g of each honey sample was dissolved with 2 mL of water then from it 1 mL of sample was taken in test tube and to it 0.4 mL of 10% aluminium chloride , 0.4 mL sodium acetate and 3 m L ethanol as added. The tubes were kept at room temperature for 30 minutes, and then absorbance was recorded at 450 nm using UV visible spectrometer. The same procedure was followed for quercetin to plot linearity.

2.3. Health Promoting Activities

2.3.1. Antioxidant Activity DPPH (1,1 Diphenyl-2-Picryl Hydrazyl) Assay

The honey samples (stock solution 1g/mL w/v) were prepared in 70% ethanol, thoroughly mixed by using vortex 5000rpm for 15 minutes and thereafter supernatant was collected for the assay. The collected supernatant was further diluted to obtain a concentration of 0.1g/mL. Similarly, the standard solution was prepared using 10mM Trolox reagent in 70% ethanol. The subsequent working solution from this stock solution were prepared from this, concentration ranging from 3.75 µM -90 µM. 100 µL of each standard solution was taken in duplicate. Then 100 µL sample was transferred to two separate wells in duplicate, one serving as sample and other serving as sample blank. Then 100 µL DPPH solution (working concentration 125 µL) was added to standard and sample wells. To sample blank wells, 100 µL 70% ethanol, was added, plates were tapped to mix properly. The absorbance was recorded at 517 nm at 5 to 10 minutes. The inhibition ratio of the sample was calculated using the formula –Inhibition ratio of Trolox (%) = (Ac-Ar)/Ac X 100 where Ac- Absorbance of 0 µM Trolox standard solution; Ar- Absorbance of 3.75- 90 µM Trolox standard solution. Similarly, Inhibition ratio of sample (%) = (Acs-As)/Acs X 100 where Acs-Blank 1-Blank 2; As- Absorbance of sample – Absorbance of sample blank.

2.3.2. Antibacterial Activity

The antibacterial activity was evaluated against six species of pathogenic bacteria (Gram negative *Escherichia coli* NCIM-2065, *Salmonella*spp NCIM-5284, *Shigella*spp NCIM-5265, *Pseudomonas aeruginosa* NCIM-2200) and (Gram positive *Bacillus cerus* NCIM-2106 and *Staphylococcus aureus* NCIM-2127). The culture was provided by Central Laboratory of Patanjali Food and Herbal Park, Haridwar, Uttarakhand ,India and the protocol followed was method described by Bhakuni et al(1974) [10].

3. Results and Discussion

All across the globe slight variation in the honey composition is observed along with differences in the biological activity. The majority of the variations are due to the botanical origin of honey and its geographical conditions and lessis due to its processing, packaging and storage conditions and environment [11]. The various physicochemical parameters present in honey are responsible for its nutraceutical and its therapeutic attributes. These parameters also reflect the quality status of honey samples.

3.1. Physicochemical Parameters

In the study the colour of litchi honey sample presented a tint of slight whitish colour although the eucalyptus, multifloral and branded honey sample were extra light amber. The variations in colour vary from, very pale yellow –amber-darkish amber- nearly black. The pH of all the honey samples ranged from 4.0- 4.36 showing acidic nature of samples, the highest pH was of branded honey sample 4.36 showing a bit less acidic nature from others. The free acidity as formic acid of branded honey sample was 0.031, however a high value was exhibited in eucalyptus and litchi honey (0.06) In a study conducted, litchi honey samples from different sources were acidic in nature [3,12]. The free acidity of the honey is influenced by the presence of low molecular mass aliphatic organic acids in equilibrium esters, lactones, and some inorganic ions, such as phosphate [13], minerals along with the botanical origin and harvest time. The pH of honey is due to these acids and different minerals. The published reports are suggestive that the pH of honey should be between 3.3-5.6 [14]. In litchi honey and multifloral, the percent moisture
content was found greater than others in study, although all the values were less than the prescribed standard value in all the honey samples. In a study low moisture content was found in litchi honey samples indicating its good storage ability however can lead to undesirable honey fermentation forming ethyl alcohol and carbon dioxide [15]. The electrical conductivity (mS/cm) of litchi honey, eucalyptus honey and branded honey sample were almost same

however the non-branded multifloral honey sample showed a little higher value (0.31). The electrical conductivity of honey is dependent on the mineral and ash content in it. However it can also be influenced by protein content, organic acids and other ions. The ash content in non-branded multifloral honey sample was higher (0.10) in comparison with other three samples. In a study conducted the litchi honey samples collected from different apiaries in Bangladesh, variation in ash content from 0.27-0.32% was found although the observed values were below 0.6% of the maximum values allowed in international standards [14]. However, in a study ash content in litchi honey was found to be 0.16%. The ash content is considered as an important quality parameter for honey [17].The carbohydrate or sugars accounts for 95-99%of honey dry matter and about 4-5% of sugars are in the form of fructo-oligosaccharides. These sugars can also affect the physical characteristics of the honeys [18]. The total reducing sugar in branded honey sample was highest (78.40%) as compared with that of other samples. The glucose and fructose content can vary even if the same variety of honey is collected from different locations. The litchi honey samples studied in Bangladesh exhibited carbohydrate content varying between 84.23-84.738%, however these results were similar and in accordance to that of honey samples from India [14,17]. As per the Codex commission the glucose and fructose content together in honey should be not less than 60% in mass ratio, and sucrose content should be not more than 5%.The sucrose from natural origin like from cane sugar, maple, beetroot can be easily added as sweeteners in honey to increase total sugar content, thus the sucrose content in honey is considered as one of the parameter to check adulteration in honey samples. [13, 19] The percent sucrose content in all the samples were within the specific limit indicative of no possible adulteration in the samples. The slight variation in the values were observed among all the samples, from 1.28-1.41, highest in the branded honey sample. Hydroxy methyl furfural in honey results from acid catalysed dehydration of the hexoses, particularly fructose. It is present in small amounts and the high levels are suggestive of adulteration in honey with acid inverted invert syrup [20]. The branded honey sample contained 24.50mg/kg HMF when tested and non-branded honey sample showed highest value among all samples 31.48. All the samples were found to be non -adulterated as the maximum limit for HMF value is<80 mg/kg. Hydroxy methyl furfural is considered as a good indicator of freshness of honey. It is formed slowly and naturally during the storage of honey and long storage period or heating of honey samples during processing or storage is responsible for increase of its content [21,22]. Proline is often regarded as a ripeness indicator of honey and, in some cases, sugar adulteration, although it represents total amino acids present in honey sample.

Table 1. *Physicochemical Properties of Different Honey Samples*

Physicochemical Parameters	Patanjali Multifloral Branded Honey	Multifloral Nonbranded Honey	Litchi Honey	Eucalyptus Honey	Acceptable Permissible limits
Colour	44	36	30	35	<50
pH	4.36	4.02	4.01	4.0	3.3-5.6
Electrical conductivity(mS/cm)	0.219	0.30	0.22	0.21	0.8(mS/cm
Total Ash Content(%)	0.06	0.01	0.079	0.08	< 0.6(%)
Acidity as formic acid	0.031	0.06	0.057	0.06	<0.2
Moisture content(%)	17.88	19.8	19.8	19.4	<=20%
Water insoluble matter(%)	0.01	0.01	0.013	0.01	0.1
Total Reducing sugar (%)	78.40	77.29	77.75	78.02	>45 (%)
Sucrose content(%)	1.41	1.28	1.32	1.31	5
Proline(mg/kg)	383.31	262.47	302.52	492.69	> 1.80mg/kg
F/G ratio	1.14	1.20	1.25	1.19	1.1-1.5% by mass
Diastase activity	13.02	10.70	11.20	23.50	>8 Schade units
HMF (mg/kg)	24.50	31.48	28.48	30.14	<80 mg/kg

**Values are mean of triplicate*

A good amount of proline content was found in all honey samples tested, although the maximum value was recorded in eucalyptus honey (492.69) and least was observed in non-branded multifloral honey sample[12,23]. The low value of proline in honey samples is found even in

non-adulterated and ripened honeys. In different studies conducted on litchi honey the variation in protein content was observed ranging from 0.52 % to even high values, in Indian samples the content was found to be lower. The protein content in honey samples is due to the different enzymes and few other derived products introduced by bees from flower nectar, however it is dependent on the type of flora visited by bees during forage [25]. Published analyses have revealed that various honeys contain 11–21 free amino acids with proline predominating [26]. The content of proline is an indication of the quality of honey and is also an indication of adulteration when it falls below a value of 183 mg/kg [16,23]. All the honey samples we studied had good proline levels of up to 383.31 mg/kg, indicating absence of adulteration. Similarly, the diastase enzyme parameter is commonly explored as indicator of honey freshness. In general, irrespective of source at least activity of 8 Schadeunits, should be present in honey. The lesser value than this is indication of long storage period or heating during processing or storage of honey must be there [22,27]. The diastase activity of honey samples analysed were in the range of 10.70-23.50 representing good quality of freshness in the sample.

3.2. Total Polyphenols Content (TPC) and Total Flavonoids Content (TFC)

The polyphenols are considered to be the potent scavengers of peroxyl radicals due to presence of high mobility of hydrogens in their molecular structure. Phenolic acids among all polyphenols are considered to be the major class of compounds responsible for colour and flavour of honey. The concentration of polyphenols in honey is considered to be as eligible parameter for quality assessment of honey [28]and considered as important honey marker. The total phenolic content and total flavonoid content in different honey samples are given in table 2as calculated through the linearity plots (figure 1,2) of gallic acid and quercetin for determining total phenolic content and total flavonoids respectively. It was found that the total phenolics in litchi honey, eucalyptus honey, non-branded honey and Patanjali honey equivalent to gallic acid (µg) were 182.42,178. 76, 156.15 and 163.25 respectively. Similarly, total flavonoids in litchihoney, eucalyptus honey, non-branded honey and Patanjali honey equivalent to quercetin (µg) were 4.34,4.34,9.10 and 4.58 respectively.

Table 2. *Total Phenolic content and Total Flavanoids in Honey samples*

SNo	Sample	Total Phenolic content (µg/g equivalent to gallic acid)	Total Flavonoid content (µg/g equivalent to quercetin)
1	Litchi honey	182.42	4.34
2	Eucalyptus honey	178.76	4.34
3	Non branded multifloral honey	156.15	9.10
4	Patanjalimultifloralhoney	163.25	4.58

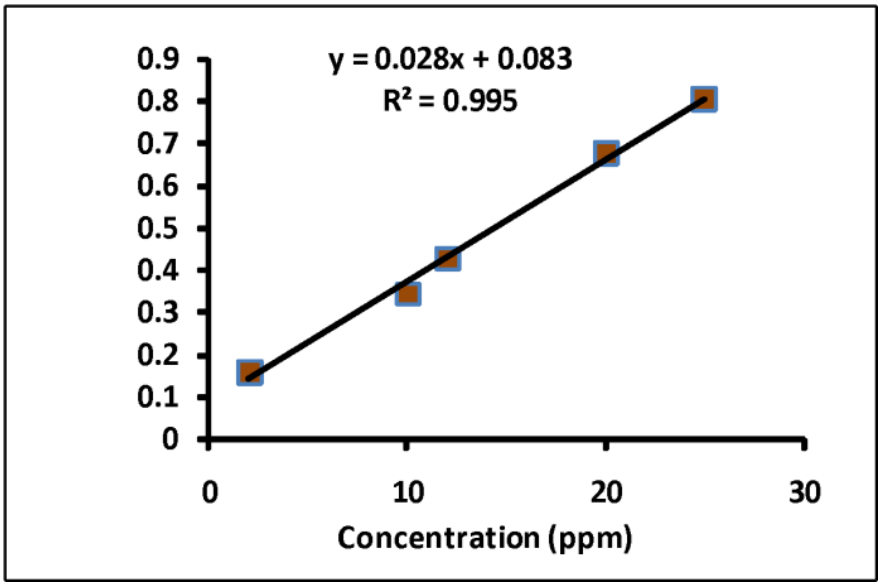

Figure 1. *Linearity plot of gallic acid for determination of TPC*

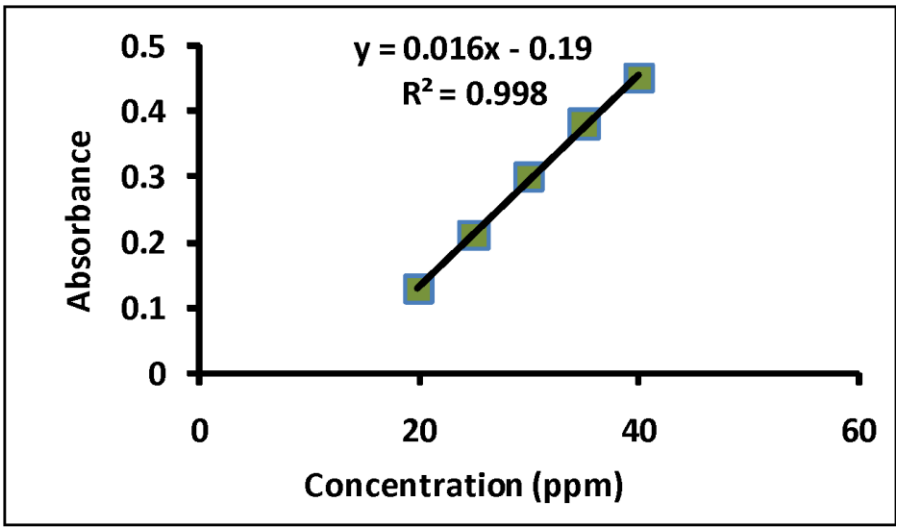

Figure 2. *Linearity plot of quercetin for determination of TFC*

3.3. Health Promoting activities

3.3.1. Antioxidant Activity DPPH (1,1-Diphenyl -2-Picryl Hydrazyl) Assay

The antioxidant activity of honey can be acclaimed due to the active biochemical compounds. It has been reported that many of the life style disease and others like cancer, cardiac problems, neurogenic diseases etc are consequence of oxidative damage. The consumption of honey as good source of antioxidant can be definitely beneficial in such situations and many researched are globally being carried out to highlight the biochemical composition, physicochemical characteristics and other pharmacological properties of honey. Till now no exact official method is found suitable to detect the antioxidant capacity of honey. The choice of the method depends on the concern of researcher, however the most commonly methods used still are FRAP (ferric reducing/antioxidant power), β-carotene bleaching assay, ORAC (oxygen radical absorbance capacity), ascorbic acid antioxidant, DPPH (free radical scavenging activity), content (AEAC), and Trolox equivalent antioxidant activity (TEAC). Each assay has its disadvantages and advantages as honey contain numerous free radical scavengers and are unable to reduce the imbalance between production of free radical and antioxidant level [29]. The results of our study are presented in table 3 showing inhibition ratio by honey samples calculated by taking trolox concentrations (3.75-90 µM). The branded Patanjlai honey exhibits (1.0g and 0.1g) 31.6 and 0.3 percent inhibition at 517 nm followed by non-branded, litchi honey and eucalyptus honey respectively.

Table 3. *Inhibition ratio of Sample (%)*

S. No	Sample	%Inhibition of sample (1.0g)	% Inhibition of sample (0.1g)
1	Litchi Honey	22.3	8.8
2	Eucalyptus Honey	20.1	8.2
3	Non branded multifloral Honey	25.1	15.0
4	PatanjalimultifloralHoney	31.6	0.3

Table 4. *Inhibition ratio of Trolox (%)*

S. No	Trolox (µM)	%Inhibition of Trolox
1	90	83.48
2	60	79.71
3	30	57.39
4	15	31.01
5	7.5	11.16
6	3.75	4.06

3.3.2. Antibacterial Activity

The antibacterial activity of honey is mainly due to active active phytoconstituents responsible for various physicochemical properties as acidity, increased osmolarity, water activity [30]. Results tabulated in table 5 show interesting zone of inhibition (Table 5).

Table 5. *Antibacterial activity of different undiluted honey samples. (Zones of inhibition mm)*

Microorganism tested	Patanjali Multifloral Honey	Multifloral nonbranded Honey	Litchi Honey	Eucalyptus Honey
Bacillus cerus NCIM2106	26	35	29	31
Staphylococcus aureus NCIM2127	32	- No inhibition	30	-No inhibition
Escherichia coliNCIM2065	41	32	26	29
Salmonella spp NCIM5248	34	33	24	23
Shigellspp NCIM5265	34	34	30	33
Pseudomaonsaeruginosa NCIM2200	- No inhibition	-26 No inhibition	- No inhibition	- No inhibition

The undiluted branded honey sample showed to be dominant in inhibiting growth of bacteria as it presented zone of inhibition for all the bacterial isolates except to *Pseudom aonasaeruginosa*. The maximum zone of inhibition was observed for *Escherichia coli* with undiluted branded honey sample. The least susceptible bacteria was *Salmonella*spp towards undiluted eucalyptus honey sample. The inhibitory activity was observed against for both gram positive and negative bacteria. It has been reported in previous studies that that honey exhibited inhibitory activity against some common gastrointestinal pathogens like *Shigelladysenteriae, Enterococcusfaecalis, Escherichia coli, Mycobacterium tuberculosis, Campylobacterjejuni, Salmonellaenterica*. In a study the zone of inhibition observed on *Pseudomonas aeroguinosa* was greater than other pathogens tested as since gram-negative bacteria are more sensitive than gram-positive bacteria. However, in our study no zone of inhibition was observed with any of the sample tested, probably the strain of the species had contributed for it, but is to be further studied. Overall the honey samples in this study showed significant antibacterial activity against gram negative and gram positive bacterial isolates except *P. aeruginosa* which reveals its efficacy of broad spectrum. In the light of this present research, it can be asserted that honey in its most concentrated form is very efficient against these isolates tested.

4. Conclusion

The various physicochemical parameters of honey samples vary according to its bee forage, geographical and climatic conditions. The present study was conducted to examine the variations in the physicochemical and health promoting activities of monofloral honey –litchi and eucalyptus honey as well as seasonal non-branded multifloral honey and Patanjali honey. It was found that Patanjali honey exhibited a good antioxidant activity and can also be considered as safe in terms of consumption as none of the samples tested were adulterated as determined by the detection of HMF, diastase and proline content. The undiluted honey samples exhibited antibacterial activity against all the bacterial isolates tested, however the none of the diluted samples showed activity against *P.aeruginosa* .The urgent need of the hour is to go for more in depth studies for all the honey samples so as to compare the honey profile all samples which would be a milestone in recognition of branded honey sample along with other locally available unifloral and multifloral sample available.

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