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A comparative study on physicochemical and antimicrobial properties of unifloral and multifloral honey samples in Northern India.

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Abstract

Honey has been used since Vedic times in India for various ailments as well as almost consumed in all Indian houses especially during winters. It has nowadays found to be promising functional food with enormous health benefits. In this study one commercially processed honey v/s three unprocessed honey (Eucalyptus , Litchi and multifloral honey from northern India) were examined. The results of different physicochemical analysis parameters of the samples ranged from pH 4.0-4.36, electrical conductivity 0.21-0.219 mS/cm, specific gravity at 27 ^{0} C 1.404-1.424, moisture content (%) 17.88-19.4,water insoluble matter (%) 0.01-0.013 ,acidity as formic acid (%) 0.031-0.06, total ash(%) 0.06-0.10, total reducing sugar(%) 77.29-78.40 , sucrose(%)1.28-1.41, proline (mg/kg)262.47-492.69,F/G ratio 1.14-1.25,diastase enzyme activity 10.70-23.50 and HMF (mg/kg) 24.50-31.48.The microbiological quality of honey samples were also examined by employing the disc diffusion method against *Bacillus cerus* ,*Staphylococcus aureus*, *Eschericiha coli*, *Salmonella* spp , *Shigella* spp and *Pseudomaonas aeruginosa*.None of the tested samples of honey(diluted or undiluted) exhibited inhibitory effect against *Pseudomonas aeruginosa*.The undiluted samples E 1 and M1 also didn't exhibited inhibitor activity against *Staphylococcus aureus* , however other samples produced zone of inhibition against all isolates in the range of 23-41mm .

Key words: Physicochemical characteristics, wound healing properties, functional food, microbial activity.

Introduction

Since ancient times the use of honey as a complementary and alternative medicine has been mentioned in Vedic texts but also in other civilizations. In this era of scientific race honey is regarded as functional food, sweetener ,antibacterial, bacteriostatic, antimicrobial , anticancer, anti-inflammatory, antitumor, prebiotic, probiotics antiseptic, antioxidant, immunomodulatory agent.[1,2]In India approximately more than 35 lakh bee colonies produce more than 1.05 lakh metric ton of honey(National Bee Board, Ministry of Agriculture). The honey collected either commercially or locally is further processed /unprocessed and can be classified accordingly. Monofloral or unifloral or varietal are single flower honey as predominance of nectar collected from single type of plant exist. The physical and chemical characteristic of honey depends on climatic and environmental conditions as well as on the biodiversity available. All across the globe this concept prevails depending on the different

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parameters. Overall all types of honey contains more than 200 bioactive compounds responsible for physicochemical properties of it as well as are important indicator of its quality.[3] The physical characteristic and medicinal attributes of honey is affected mainly by its chemical composition, however the physical appearance of it varies accordingly with methods of extraction, processing, packaging and preservation techniques.[4] A very little research has been conducted on the antibacterial properties of honey in India , although multiple studies for the same is carried all over the world.[5,6] Many researchers propose that the presence of active phytoconstituents like , phenolic compounds, organic acids, volatile compounds, leading to different physicochemical properties as acidity, increased osmolarity, water activity are responsible for antibacterial activity of honeys.[7-10]The major area of interest in this study is to find the variation among monofloral and multifloral honey with respect to the various biochemical, physicochemical and health promoting activities. This comparative study is the need of hour so that the medicinal and quality attributes can be highlighted after investigations and thus meets a void in research.

Materials and Method

Sufficient samples were procured such that all experiments during the study were performed with same sample batch. The different honey samples were sourced mainly from the local beekeepers (monofloral honey-Eucalyptus and Litchi honey);seasonal multifloral honey and one branded honey –Patanjali honey). The duration of collection of samples were done as per the seasonal fruiting period of mononfloral honey and within same duration other samples were procured so as to maintain uniformity. The samples used were without any preservative during the entire study. All the methods used are in accordance with the national and international standards routinely followed in Honey industries.

Preparation of honey samples

All honey samples were prepared according to the guidelines provided by IS Standard, Annexure J, Clause6.1.All samples were free from suspended solids , granulation and any form of crystallization. A clean sterile glass rod was used to thoroughly mix the samples to ensure homogeneity prior to use.

Physicochemical and Biochemical Characterization of Honey Samples

The physicochemical parameters were determined according to the methods described in ‘Indian Honey Specification’ by the –FSSAI 2020.All the samples were taken in triplicate.

Colour: The colour of all samples of honey was analysed 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. The samples were 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 Pfund values.

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pH: The procedure described by AOAC (962.19:1990) was used to determine pH (HI 9025-HANNA) with a sample of honey diluted in 10% of distilled water.

Electrical Conductivity: The method to determine electrical conductivity of honey samples used was as described by World Network of Honey Science .The method is valid for the determination of the electrical conductivity of honey in the range 0.1 - 3 $mS.cm^{-1}$. The results are expressed in milli Siemens per centimetre (mS/cm).

Moisture Content: The moisture in honey samples was detected by refractometer method as per standard provided by International Honey Commission 2009.

Water Insoluble Content :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.

Acidity (as Formic acid): The acidity , is the sum of all the free acids expressed in meq/kg of honey .10 g sample was dissolved in 75 ml of carbon di oxide free water and titrated against standard solution of sodium hydroxide using phenolphthalein as an indicator.

Total Ash Content: The ash content was determined by heating 5 g of honey with few drops of pure olive oil to prevent spattering in a muffle furnace at 600°C +- 20 ^{0} C till ash is obtained . After cooling, the ash content was calculated.

Total Reducing sugar : 1 g of prepared sample of honey was placed in 250 ml volumetric flask and diluted with 150 ml of water and thoroughly mixed to make volume to 250 ml with water. 5 ml each of copper sulphate solution and potassium sodium tartrate (Rochelle salt) solution was added to 12 ml of honey solution and sample heated to boiling over an asbestos quaze. 1 ml of methylene indicator was used and while keeping the solution boiling titration was complete till colour changed from blue to red.The total reducing sugar vs percent by mass was calculated using a standard formula –

250 x 100 x S/ H x M where S = strength of copper sulphate solution; H= volume in ml of honey solution required for titration; M=mass in g of honey

Sucrose content : To 100 ml of stock honey solution 1 ml of concentrated HCl was added and the solution was to near boiling and kept aside overnight. This inverted honey solution was neutralized with sodium carbonate and total reducing sugar was determined.

Amino acid –Proline : The determination of proline was done according to the method provided by IHC 2009.

Fructose /Glucose ratio : 50 ml of honey solution was pipette out in a 250 ml stoppered flask. Then 40 ml of iodine solution and 25 ml of sodium hydroxide solution was added to it

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and stoppered the flask and kept in dark for 20 minutes.The sample was further acidified with 5 ml of sulphuric acid and titrated quickly with excess of iodine against standard thiosulphate solution . Results were calculated using formula z/y wher z= true fructose , percent by mass and y = true glucose , percent by mass.

Diastase Enzyme activity: The diastase enzyme activity was determined by the Phade base method(Phadebas® Honey Diastase Test, Magle AB).

Hydroxymethylfurfural(HMF) : The method followed used for HMF determination was as per guidelines provided in Indian Standard for extracted honey (2002). 10 g of honey sample was dissolved without heating in 20 ml oxygen free distilled water and made the volume upto 50 ml (honey solution).The sample was tested after preparation without delay.The photometric determination was carried out by adding 5.0 ml p-toluidine solution and 1 ml barbituric acid solution in the sample . The extinction of the sample is read against the blank at 550 nm using a 1 cm cell, immediately after the maximum value is reached . An equation by which result were worked out is mg/l00 g HMF = Absorbance ×19.2 Thickness of Layer. Results are expressed as mg HMF/kg honey.

Antibacterial activity : The antibacterial activity of all the four samples of honey in triplicate was tested and evaluated against six species of pathogenic bacteria commonly encountered in human infections. The culture was provided by Central Laboratory of Patanjali Food and Herbal Park, Haridwar , Uttarakhand ,India. The antibacterial activity was carried out using the standard proptocol .[11,12]The test organism used for antimicrobial activity were gram negative bacteria –(*Escherichia coli* NCIM-2065,*Salmonella* spp NCIM-5284,*Shigella* spp NCIM-5265,*Pseudomonas aeruginosa* NCIM-2200) gram positive *Bacillus cerus* NCIM-2106 and *Staphylococcus aureus* NCIM-2127. The diameter of zone of inhibition including that of well was measured using Vernier calliper (Mitutoyo model CD 12 PSX) .The control plates were prepared using sterile distil water. All plates were incubated for 24 hours at 30^0 C and then zone of inhibition were measured.

Results and Discussion `

The results of different physicochemical parameters are given in table 1.The colour of the eucalyptus, multifloral and branded honey sample were extra light amber however litchi honey sample presented a tint of slight whitish colour. The variations in colour in different honey samples all across the globe shows variations due to different botanical origin, geographical locations resulting in slight variation in physicochemical parameters, however the colour of honey varies from very pale yellow –amber-darkish amber- nearly black. Free acidity is a parameter helping to assess the deterioration level of honey, being its limit established as 50 meq/ kg .[13,14] The pH of honey is due to the presence of different acids and minerals. 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) . 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 various published reports are suggestive that the pH of honey should be between 3.3-5.[15] The organic acids present in honey are mainly responsible for the characteristic aroma, flavour, acidity , pH and electrical conductivity and can also be used for the determination of its freshness, and its authenticity .[16] The percent moisture content was found greater in litchi and multifloral. Honey samples however it was less (17.88) in branded honey sample , although all the values were less than the prescribed standard value in all the honey samples(Figure1).

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Table1 : Physicochemical Properties of Different Honey Samples.(Values are average of triplicate)

Test Parameters	E1-Eucalyptus Honey	L1-Litchi Honey	M1-Multifloral nonbranded Honey	B1-Multifloral Branded Honey
Colour	35	30	36	44
pH	4.0	4.01	4.02	4.36
Electrical conductivity(mS/cm)	0.21	0.22	0.30	0.219
Total Ash Content(%)	0.08	0.079	0.01	0.06
Acidity as formic acid	0.06	0.057	0.06	0.031
Moisture content(%)	19.4	19.8	19.8	17.88
Water insoluble matter(%)	0.01	0.013	0.01	0.01
Total Reducing sugar(%)	78.02	77.75	77.29	78.40
Suucrose content(%)	1.31	1.32	1.28	1.41
Proline(mg/kg)	492.69	302.52	262.47	383.31
F/G ratio	1.19	1.25	1.20	1.14
Diastase activity	23.50	11.20	10.70	13.02
HMF (mg/kg)	30.14	28.48	31.48	24.50

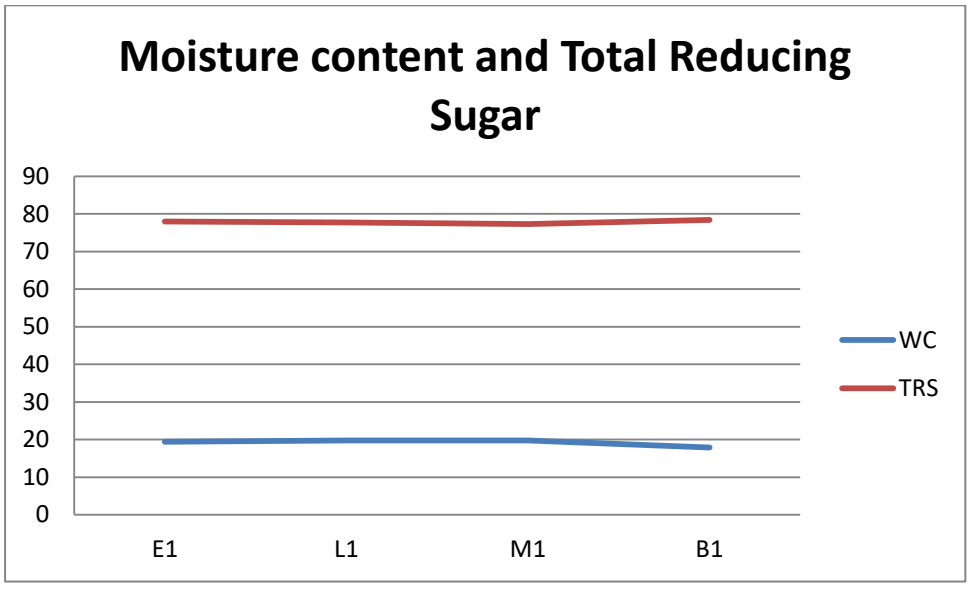

Figure 1: Water content and Total Reducing sugar in different honey samples.

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The maximum limit of moisture content recommended by International quality regulations is <=20% (Codex Alim 2001).[14] The moisture content in honey is an important parameter affecting its quality and it varies from 15-23% as depends upon bee variety, bee colony strength ,air temperature and humidity in bee hive ,climatic conditions ,botanical origin ,and processing and storage conditions of honey samples.[17,18] 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.[18] The electrical conductivity (mS/cm) of litchi honey, eucalyptus honey and branded honey sample were almost same (0.22,0.21,0.219 respectively) however the non-branded multifloral honey sample showed a little higher value (0.31) (Figure 2). 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 nonbranded multifloral honey sample was higher (0.10) in comparison with other three samples(0.06-0.08).The ash content in honey is mainly composed of minerals like sodium, potassium, magnesium and calcium which play an important role in different biochemical and physiological activities of humans. The high ash content may indicate an excess of inorganic substances from external contaminants such as equipment or during handling of honey as well as due to environmental pollution .Thus it is considered as an important quality parameter for honey .[19] 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.[15] The observed ash content is dependent on the environmental as well as geographical location and also the material collected by bees during foraging on flora. 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.[20] The total reducing sugar in branded honey sample was highest (78.40%) as compared with that of other samples (77.29-78.02%) (Figure1).Different studies have been conducted to analyse the sugar profile of different honey. 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.[15,19] However the fat content was very low (0.002-0.003%) in these samples. It has been reported that chestnut and acacia honey have a very high fructose content whereas rapeseed honey contains a higher glucose content.[17,18] 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. [21] 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(Figure3).Hydroxymethylfurfural 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 .[22] The branded honey sample contained 24.50mg/kg HMF when tested and nonbranded honey sample showed highest value among all samples 31.48 . All the samples were found to be nonadulterated 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.[23,24] Proline is often regarded as a ripeness indicator of

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honey and, in some cases, sugar adulteration, although it represents total amino acids present in honey samples. 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. A minimum value of 180 mg/ kg of proline is proposed for a pure honey however, considerable variation in it occurs according to the type of honey and low values can be found even in non-adulterated and ripened honeys.[13,25] 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.[19,26] Published analyses have revealed that various honeys contain 11–21 free amino acids with proline predominating.[5] 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 .[18] All the honey samples we studied had good proline levels of up to 183 mg/kg, indicating absence of adulteration. Proline is the most abundant amino acid in honey and is used as a standard to quantify amino acid content.In honey diastase enzyme (diastase units) is a parameter commonly explored as indicator of honey freshness . In general irrespective of source at least activity of 8 Schade units, 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 .[24,27] The diastase activity of honey samples analysed were in the range of 10.70-23.50 representing an good quality of freshness in the sample.

Figure 2 :Electrical conductivity (mS/cm) and Total ash content of different honey samples.

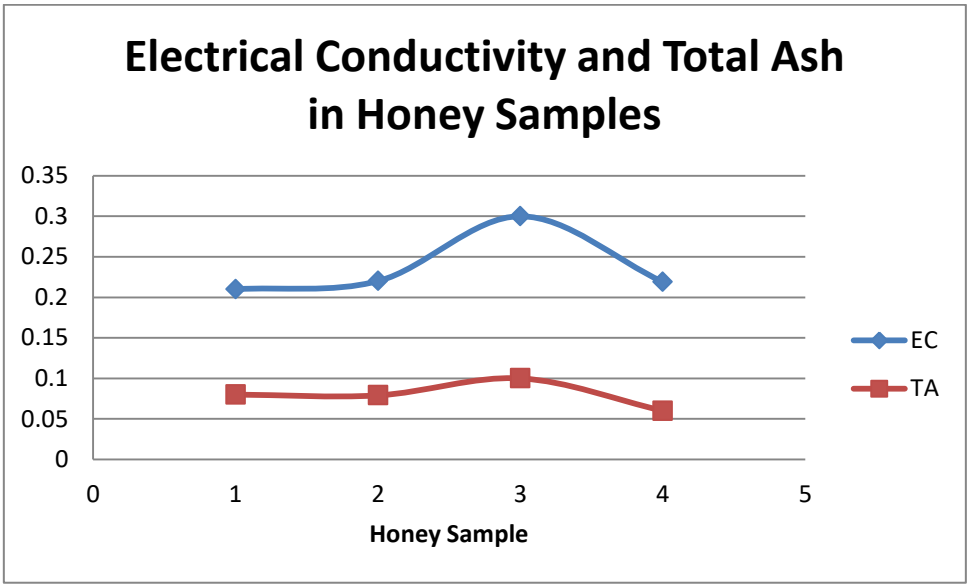

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Figure 3: Sucrose content in different Honey Samples

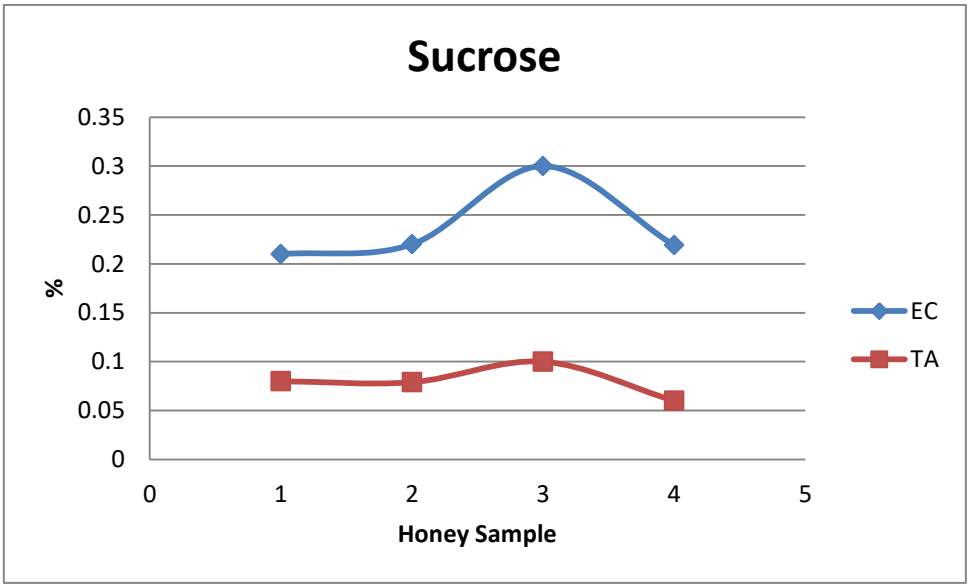

Figure 4 : Proline content in different honey samples.

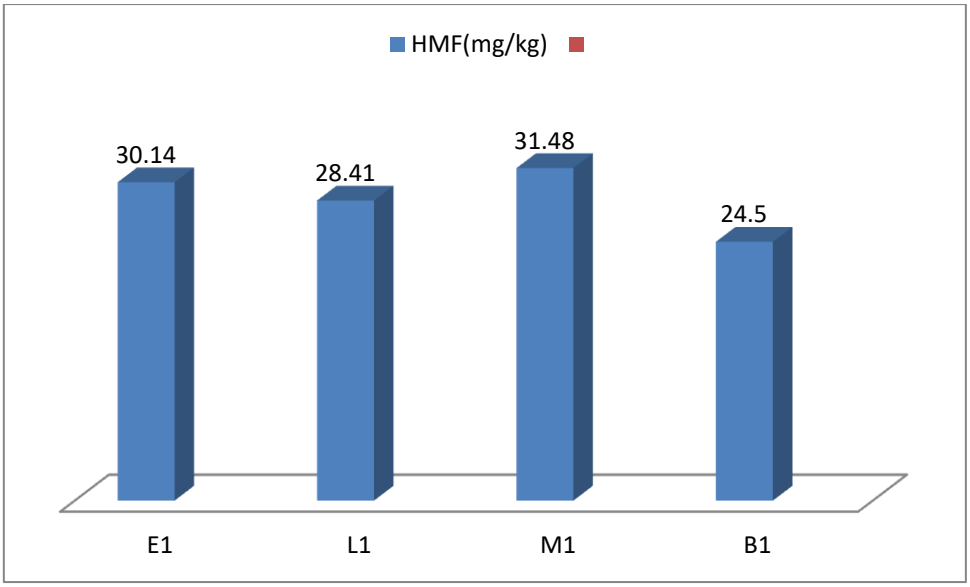

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Figure 5 : F/G ratio in different honey samples.

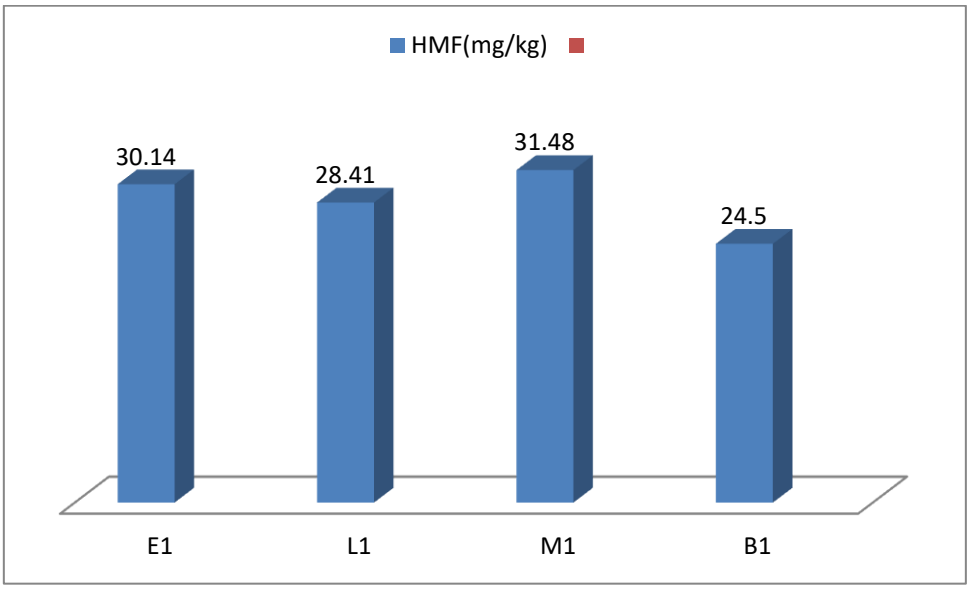
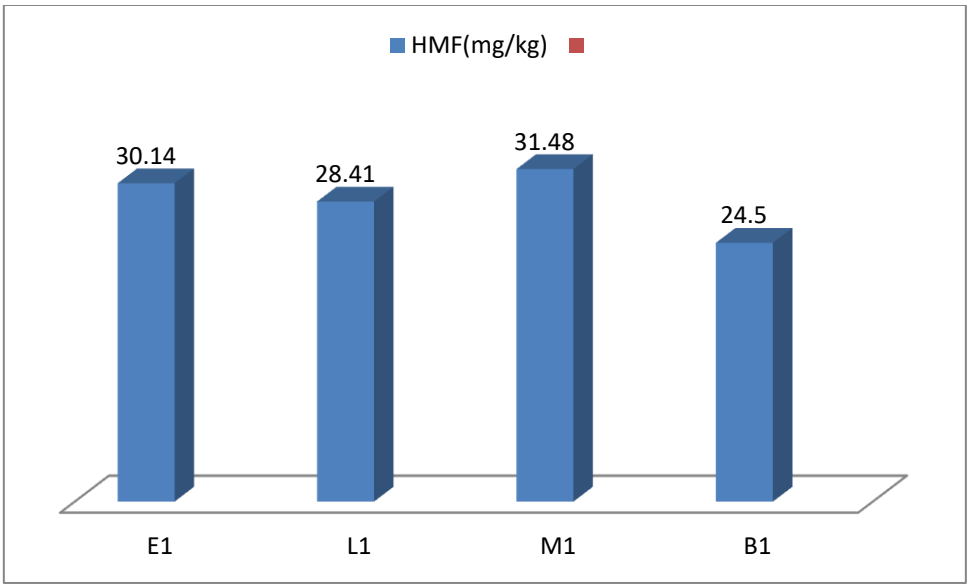


Figure 6: Diastase in different honey samples.



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Antibacterial activity

Results tabulated in table 2 show interesting zone of 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 *Pseudomaonas aeruginosa* . 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.It has been found that at low pH of honey the growth of the bacteria is inhibited , however other factors like hydrogen peroxide, sugar content enzymes etc are also reported to inhibit bacterial growth.

Table 2 –Antibacterial activity of different undiluted honey samples.(Zones of inhibition mm).

Microorganism tested	E1-Eucalyptus Honey	L1-Litchi Honey	M1-Multifloral nonbranded Honey	B1-Multifloral Branded Honey
Bacillus cerus NCIM2106	31	29	35	26
Staphylococcus aureus NCIM2127	-No inhibition	30	- No inhibition	32
Escherichia coliNCIM2065	29	26	32	41
Salmonella spp NCIM5248	23	24	33	34
Shigell spp NCIM5265	33	30	34	34
Pseudomaons aeruginosa NCIM2200	- No inhibition	- No inhibition	-26 No inhibition	- No inhibition

The antibacterial properties of honey produced by *Apis mellifera* have been extensively studied and the concentration of honey tested for activity ranged from 100% - 25% (v/v). The inhibitory activity was observed against for both gram positive and negative bacteria .The well diffusion method is considered better than any other method to detect antibacterial activity as in this the minutes particle in honey tend to migrate more easily the action of the

micro-organisms . It has been reported in previous studies that that honey exhibited inhibitory activity against some common gastrointestinal pathogens like *Shigella dysenteriae ,Enterococcus faecalis , Escherichia coli, Mycobacterium tuberculosis, Campylobacter jejuni,Salmonella enterica*. The development of bacterial biofilms formed by *Streptococcus pyogenes* and *Pseudomonas aeruginosa* were also inhibited by honey samples.[7,8,9,10] 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.

Conclusion

The in depth studies for all the honey samples should be further analysed along with antioxidant properties and other characteristics 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 As the antibacterial activity of honey depends on its physical and chemical factors so the comparative analyses on its physicochemical properties should be more extensively conducted.

Conflict of interest : The authors declare that they have no conflict of interest.

References

1. Al-Waili, N., Salom, K., Al-Ghamdi, A., & Ansari, M. J. Antibiotic, pesticide, and

ISSN PRINT 2319 1775 Online 2320 7876
, Journal Volume 13, Iss 01, 2024

microbial contaminants of honey: Human health hazards. *The Scientific World Journal, 2012,* 1–9

2. Cornara, L., Biagi, M., Xiao, J., and Burlando, B. Therapeutic properties of bioactive compounds from different honeybee products. *Front. Pharmaco, 2017,* 8, 412.
3. Azonwade, F. E., Paraïso, A., Agbangnan Dossa, C. P., Dougnon, V. T., N'tcha, C., Mousse, W., & Baba-Moussa, L. Physicochemical characteristics and microbiological quality of honey produced in Benin. *Journal of Food Quality* ,2018, https://doi.org/10.1155/2018/1896057
4. Bontempo, L., Camin, F., Ziller, L., Perini, M., Nicolini, G., & Larcher, R. Isotopic and elemental composition of selected types of Italian honey. *Measurement*, 2017 ,*98*, 283-289.
5. White RJ, Cutting K, Kingsley A. Topical antimicrobials in the control of wound bioburden, Ostomy. *Wound Manage* 2006;52:26e58
6. Molan PC. Re-introducing honey in the management of wounds and ulcers e theory and practice. Ostomy. *Wound Manage* ,2002;48:28e40

7. Alvarez-Suarez JM, Giampieri F and Battino M. Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. *Curr. Med.Chem, 2013,.20:621-38.*

8. Cushnie TP and Lamb AJ. Antimicrobial activity of flavonoids.*Int .J.Antimicrob.Agents,*2005,26:343-56.

9. Lin SM, Molan PC and Cursons RT. The controlled in vitro susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey. *Eur. J. Clin. Microbiol. Infect. Dis, 2011,* 30: 569-74.

10. Voidarou C, Alexopoulos A, Plessas S, Karapanou A, Mantzourani I,Stavropoulou E, Fotou K, Tzora A, Skoufos I and Bezirtzoglou E. 2011. Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobe,2011,* 17: 375-9.

11. Sheikh, D., Zaman, S., Naqvi, S. B., Sheikh, M. R., & Ali, G. Studies on the antimicrobial activity of honey. *Pak J Pharm Sci*, 1995,*8*(1), 51-62.

12. Bhakuni D.S., BittnerM.,Marticarena C.,SilvaM.and Weldt E.Screening ofChilean plants for antibacterial activity.*Lloydia* ,1974,37:621-632.

13. Mărgăoan, R., Topal, E., Balkanska, R., Yücel, B., Oravecz, T., Cornea-Cipcigan, M., & Vodnar, D. C. Monofloral honeys as a potential source of natural antioxidants, minerals and medicine. *Antioxidants*, 2021,*10*(7), 1023.

14. Codex Alimentarius Commission (2001). *Codex Standard for Honey, CODEX STAN 12–1981*.

15. Islam A, Khalil I, Islam N, Moniruzzaman M, Mottalib A, Johnson, R. M. Honey bee toxicology. *Annual Review of Entomology*, 2014, *60*, 415–434.

ISSN PRINT 2319 1775 Online 2320 7876
, Journal Volume 13, Iss 01, 2024

16. Kolayli, S., Sahin, H., Can, Z., Yildiz, O., & Sahin, K. Honey shows potent inhibitory activity against the bovine testes hyaluronidase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2016 ,*31*(4), 599-602.
17. Bobis, O., Moise, A. R., Ballesteros, I., Reyes, E. S., Durán, S. S., Sánchez-Sánchez, J., ... & Alvarez-Suarez, J. M. Eucalyptus honey: Quality parameters, chemical composition and health-promoting properties. *Food chemistry*, 2020, *325*, 126870.
18. Bogdanov, S., Ruoff, K., & Persano-Oddo, L. Physico-chemical methods for the characterisation of unifloral honeys: A review. *Apidologie*, *2004*, *35*, S4–S17.
19. Saxena, S., Gautam, S., & Sharma, A. Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry*, 2010, *118*(2), 391-397.
20. Dobre, I., Georgescu, L. A., Alexe, P., Escuredo, O., & Seijo, M. C. Rheological behavior of different honey types from Romania. *Food Research International*, *2012*,*49*(1), 126–132.
21. Perez-Arquillue, C., Conchello, P., Arino, A., Juan, T., Herresa, A. Quality, evaluation of Spanish rosemary (Rosomarinus officinalis) honey. *Food Chemistry* ,1994,51, 207–210.
22. Doner, L. W. The sugars of honey—a review. *Journal of the Science of Food and Agriculture*, 1977,*28*(5), 443-456.
23. Khalil MI, Sulaiman SA, Gan SH. (2010) .High 5- hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. *Food Chem Toxicol* ,2010, 48:2388–2392.
24. Yucel, Y., & Sultanoglu, P. Characterization of honeys from Hatay Region by their physicochemical properties combined with chemometrics. *Food Bioscience*, 2013, *1*, 16–s25.
25. Wu, H. C., Shiau, C. Y., Chen, H. M., & Chiou, T. K. . Antioxidant activities of carnosine, anserine, some free amino acids and their combination. *Journal of Food and Drug Analysis*, 2003, 11, 148–153.
26. Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., Kayacier, A. Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*, 2013, *46*, 124–131.
27. Tosi, E., Martinet, R., Ortega, M., Lucero, H. A., & Ré, E. Honey diastase activity modified by heating. *Food Chemistry*, 2008, *106*(3), 883-887.