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RESEARCH ARTICLE

EVALUATION OF PHYSICOCHEMICAL AND ANTIMICROBIAL PARAMETERS OF HONEY FROM DISTRICT NEELUM, AJ&K

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ABSTRACT

Honey is the natural sweet substance produced by bees. Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market. In the present study, fifteen honey samples were collected from five targeted sites of district Neelum namely Kathan peer, Ashkot, Nakdar, Lasva and Lawat. The samples were then analyzed for physicochemical and antibacterial properties. The obtained values of physicochemical parameters like pH, electrical conductivity, moisture, acidity, sucrose and total sugar were compare with international standered provided by the Codex Alimentarius. All the values were within the prescribed limit except for moisture. 13 samples exceed the approved value of moisture content indicating the moist environmental conditions and improper handling. Honey showed effective antibacterial activity against *Klebsiella pneumoniae, Staphylococcus epidermidis, Serratia marcesscens, Staphylococcus aureus* and *Streptococcus pyogenes* which is an evidence that honey is a therapeutic agent being used since ancient time throughout the world.

Keywords- : Honey, Acidity, Antibacterial, Physicochemical

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INTRODUCTION

Honey is a naturally assimilated carbohydrate product formed by an insect belonging to the genus *Apis,* order Hymenoptera, locally recognized as honeybee (1, 2). It is a sticky and viscous solution with a content of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and minor quantities of amino acids, enzymes and vitamins as well as other substances like phenolic antioxidants (3, 4). The minor constituents are known to have distinctive nutritional or medicinal properties (4) while the major constituents are nearly the same in all honey samples.

The precise chemical composition and physical properties of natural honey differ according to the plant species on which the bees forage (4, 5) and also according to climatic conditions and vegetations which are important factors that can affect the various properties of honey.

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market. Honey production and processing has the potential to become a major foreign exchange earner for azad Kashmir. Honeybees have a great deal to offer as far as rural items and biological community services. Honeybees are the most important pollinators which may expand yield 10 to 20 times (6) of different products.

The development of a viable honey production and processing industry in the district may benefit from biochemical analysis of natural honey samples obtained from different locations of the study area in order to ascertain product quality vis-à-vis international standards. Therefore this paper reports the physicochemical and microbioological properties of honey.

STUDY AREA

District Neelum was selected as study area. The District is located north-east of district Muzaffarabad. It situated between 73° to 75° E longitude and 32° to 35° N latitude at a height of 900-6325 meters above sea level. District Neelum is the largest region of Azad Kashmir comprising area of 3737 km. The environment is calm and having extremely cool winters (normal 0 to 4 ºC) and direct summers (normal temperature upto 30 ºC). Territory encounters long serious winters and a little mellow summers.

METHODOLOGY

Sample collection and preparation

Fifteen samples from five different sites namely Kathan peer, Ashkot, Nakdar, Lawat, and Lasva were collected in month of September to November (2018).Three samples were collected from different areas of each site. Sampling was done by random sampling method and samples were packed in sealed glass bottle with no preservative.

250g honey was mixed in 250ml of distilled water in 500ml eppendorf. The honey solution was centrifuged at 1500rpm for 15 minutes. Samples were then filtered through Whatman filter 1 to remove all the suspended contaminents. Prepared samples were preserved in sealed eppendorf,and stored at room temperature (25°c-30°c) for analysis. Samples of honey were prepared with distilled water as honey has best dissolution with dissteled water.

Physicochemical analysis

pH and electrical conductivity: The pH values and electrical conductivities of the honey samples were measured at 28 ± 2° C using pH and conductivity meters. The pH of the honey samples were determined in a 10% aqueous honey solution using a digital pH meter after it was calibrated at pH 4.0 and 7.0 using standard buffer solutions

Moisture contents: The water percentage of each nectar sample was measured by beneath Equation. In a pre-measured aluminum dish around 5 g of the honey sample was set. At that point sample was dried to steady weight in a stove at 105°C for 4 hours (7)

Moisture percentage = w1-w2/w1-w2 X 100

W1= fresh sample weight + dish (g) and w2 = dried sample weight + dish (g).

Acidity: Acidity was find by mixing 10 g of honey with 75 mL of water and then the solution was titrated with 0.1 M NaOH solution until 8.6 pH obtained (8,9). Acidity in milliequivalens/kg of honey was calculated

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using AOAC 1990 official method 962.19 as follows
Acidity (meq/kg) = volume of 0.10 M NaOH x 10 (2) Where, the dilution factor of honey sample is indicated by 10.

About ruddy orange shading was estimated at 540 nm. The Sucrose content in each nectar test was estimated utilizing 20 percent (w/v) solution by refractometric strategy. Aggregate of sucrose and other sugars results

(Oxide: CM1) and nutrient agar (Oxide: CMOO3) were used for culturing bacteria. A loop full of strain is inoculated in 25 ml of nutrient broth medium and incubated at 37°C on a rotary shaker for 24 h to activate pathogens. This culture was dissolved in freshly prepared nutrient agar medium (NAM) at 45°C and was setteled into the sterilized Petri dishes.To solidification the culture all Petri dishes were kept in laminar flow at room temperature. Three wells of 5 mm diameter were made in each plate using a sterilized micropipette tip of 1ml and sterilized needle was used for the removal of agar plug. Approximately 30 µl of each crude

RESULTS AND DISCUSSION

Physicochemical parameters: Table 1 shows the results of some physicochemical parameters of honey samples from the different sites of study area. The pH values ranged from 3.42 to 4.21. Sample from ashkot had minimum pH value of 3.42 whereas highest pH was measured in sample from nakdar.
Moisture contents ranged from 18.01 to 23.04. Values of maximum samples were not in accordance with the international standered (≤19 percent). The acidity of samples lie between 10.04 to 20.01 meq/kg that meet the limit of international standered.
Conductivity was within the range of 0.14 to 0.62 Ms/cm. The lowest value of 0.14 Ms/cm was shown by lasva honey and lawat honey had highest conductivity of 0.62 Ms/cm.

Sucrose and total sugar: Honey solution in water (1.0mL of 1.0mg/mL) was blended with 1.0mL of DNSA (3,5-dinitrosalicylic acid) (Sigma Aldrich, USA), put the solution in water bath for ten minutes until warmed, and the absorption spectra of coming
in the total sugar substance in g/100 g of nectar (10).

Antibacterial analysis

The antimicrobial property was detected by the agar-well diffusion method (11). Nutrient broth media
extract and control solvent samples were placed in each prepared wells and placed at 37°C for 24-48 h. The all solvents were also used as a negative control. Microbial growth was determined by measuring the diameter of zone of inhibition after 24 h in millimeter.

Test microorganism

For current research work, five bacterial pathogens were used and collected from Department of Zoology, Azad Jammu and Kashmir University, Muzaffarabad, Pakistan. The bacterial strains include, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Serratia marcesscens* , *Staphylococcus aureus* and *Streptococcus pyogenes*.
Total sugar contents of all the honey samples were within the international standered (not less than 60g/100g). Sucrose percentage ranged betwee 5.62 to 9.23.kathan peer honey having highest percentage of 9.23 and nakdar honey having lowest 5.62 percent.

Antimicrobial activity: To study the antimicrobial activity in honey the gel diffusion method was applied. Honey samples showed variable degrees of inhibition against the cultures swabbed (table 3). Lawat honey acted best against *Staphylococcus epidermidis*. All other samples also show remarkable antibacterial activity against *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Serratia marcesscens, Staphylococcus aureus* and *Streptococcus pyogenes*.

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Table 1: Physicochemical parameters of honey samples from the five study sites

Parameters	Kathan peer	Ashkot	Nakdar	Lawat	Lasva
pH	3.82 ± 0.14	3.68 ± 0.02	4.01 ± 0.04	3.75 ± 0.05	3.64±0.12
Moisture (%)	19.6 ± 0.18	21.36± 0.15	19.70±0.02	21.71±0.08	21.04±0.02
Acidity(meq/ kg)	16.06± 0.19	14.03± 0.15	13.39± 0.12	20.69± 0.02	13.52± 0.13
Conductivity (Ms/cm)	0.51± 0.14	0.49± 0.12	0.23± 0.02	0.16± 0.9	0.18± 0.18
Total sugar (%)	81.36± 0.16	78.67± 0.08	83.37± 0.19	81.15± 0.22	79.71± 0.26
Sucrose (%)	6.72± 0.34	8.22± 0.16	8.24± 0.12	7.54± 0.22	8.64± 0.24

values presented are mean ± SD of three determinations. Mean values with different superscript along rows are significantly different (P<0.05

Table 2: Physicochemical parameters of 15 honey samples from targeted sites

Parameters	Mean± SD	Range of values (Min–Max)	Limits of Int'l Standards	Samples outside the Limits of International Std.
pH	3.80± 0.18	3.42-4.21	No fix limit	-
Moisture (%)	20.48± 0.09	18.01-23.04	≤19 percent	13 samples
Acidity(meq/kg)	15.54± 0.15	10.04-20.01	Not>50meq/ kg	-
Conductivity(M s/cm)	0.31± 0.02	0.14-0.62	Not>0.8mSm	-
Total sugar (%)	80.85± 0.26	76.01-85.14	Not<60g/10g	-
Sucrose(%)	7.87± 0.19	5.62-9.23	Not>10g/10g	-

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Table 3: Inhibition zones (in mm) of honey samples

Samples	*S.Aureus*	*K.Pneumonia*	*S. Marcesscens*	*S.Epidermidis*	*S. Pyogenes*
Kathan peer	19± 0.10	20± 0.13	20.6± 0.26	19.3± 0.12	18.6± 0.14
Ashkot	21± 0.12	17± 0.21	16.3± 0.16	19.3± 0.12	19.6± 0.16
Nakdar	19.5± 0.12	19± 0.12	20.3± 0.1	20.3± 0.13	20.6± 0.12
Lawat	22.3± 0.2	18.5± 0.12	18.3± 0.18	23.3± 0.14	20.3± 0.12
Lasva	19.5± 0.10	18± 0.14	18.6± 0.12	20.6± 0.02	19.6± 0.22

values presented are mean ± SD of three determination

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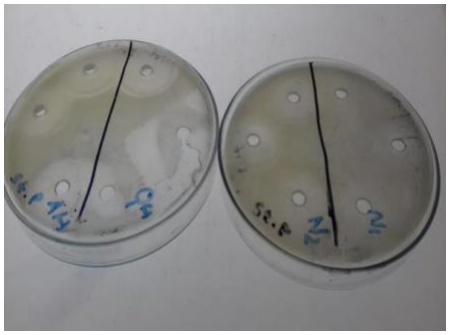
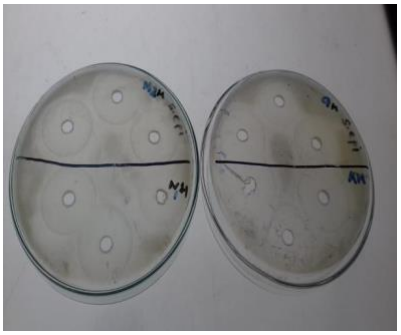
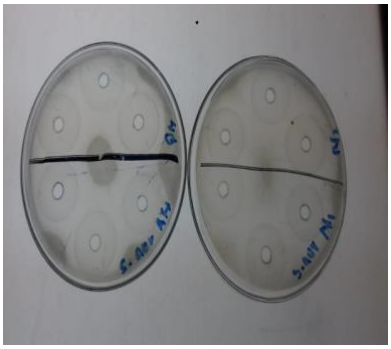


(a) antibacterial activity of *Streptococcus pyogenes*

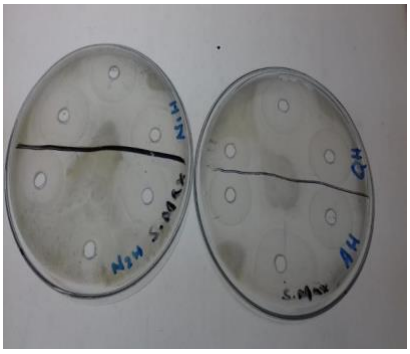
(b) antibacterial activity of *klebsiella pneumoniae*



(c) antibacterial activity of *Staphylococcus epidermidis*



(d) Antibacterial activity of *Staphylococcus aureus*



(e) Antibacterial activity of *Serratia marcesscens*

Fig. 1.Antibacterial activity of honey samples against (a) *Streptococcus pyogene,* (b) *Klebsiella pneumoniae*, (c) *Staphylococcus epidermidis*, (d) *Staphylococcus aureus and (e) Serratia marcessce*

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The pH values of the honey samples from the different States were not significantly different from each other; the values ranged from 3.42 and 4.21 with an average of 3.8 pH values ranging 3.6 and 4.32 were reported for Argentine, Spanish, and Italian honeys (12,13). The honey samples analysed in the study are acidic with pH <7. According to Leveen *et al.*, (14) the acidic pH of honey is desirable because acidification promotes wound healing by causing oxygen release from haemoglobin.

The moisture and sugar percentages of honey are entirely associated (15) the Moisture percentage relies upon many factors, for example, extraction season, level of maturation came to in the hive and environmental conditions (16). The dampness content in Pakistani honey estimated by Ahmed *et al.,* (17) was extended between 10.1and 17.3 %.The moisture contents of study samples varies between 18.01 to 23.04 % that is not in accordance with the moisture limit (≤19 percent) approved by the Codex Alimentarius (18). 13 samples have higher percentage than prescribed limit. The higher moisture percentage may be due to moist environmental conditions of area, pre-ripened extraction of honey or bad storage conditions. The honey having higher water content had brought down total sugar and vise versa.. However the total sugar identified is under considerable range between 76.01-85.14 %.Total sugar percentage is under the international norm specifies by European Council (not <60g/100g). Sucrose values ranged between 5.62 to 9.23 % with an average of 7.87%.

The conductivities of the honey samples analysed in this study varied between 0.14 and 0.62 with an average value of 0.31. The international norm specified by both Codex Alimentarius Commission and European Council (EU) established values of<0.8 mS/cm for blossom honey or blends (mixtures) of blossom honey and >0.8 mS/ cm for honeydew honey. The conductivity data in this study shows that all the samples fall within the range required by the international standard. The results also indicate that all the samples from the different locations are of floral botanical origin. Conductivity is good criterion for determining botanical origin of honey and today it is determined in routine honey quality control instead of the ash content (19).

Writing sources demonstrate that antibacterial movement of nectar impressively relies upon the botanical source (20) thus the honey could be recognized by their transcendent plant organization. It is intriguing to take note of those floral sources of some honey was very comparable in any case, their antimicrobial movement was unique. In our study antibacterial activity of honey samples against five different pathogens *viz*., *Serratia marcesscens*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. was detected. All the samples show high degree of antibacterial activity. Highest activity was being investigated against *S. Epidermidis* having diameter inhibition zone 23.3mm whereas lowest is being shown against *S. Marcesscens* having diameter inhibition zone 16.3mm.

CONCLUSION

Present study concludes that to ensure improved quality and quantity of honey, implementation of advanced methods of apiculture is necessary. The current study also testifies the honey obtained from area has high antibacterial activity, emphasizing its importance in medicine.

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