SXC JOURNAL
Volume 1, 2024

Physicochemical and Antibacterial Properties of Nepalese Honey

Aayushma Shakya, Aashish Dhakal, Aastha Tamang, Aakriti Adhikari, and Kamil Prajapati*
Department of Microbiology, St. Xavier's College, Kathmandu, Nepal
*Email: kamil@sxc.edu.np**

ABSTRACT

Nepal has a variety of honey and bee products that are dependent on floral sources available in a particular geographical region. The purpose of this study was to compare the physicochemical and antibacterial properties for the quality analysis of different honey samples. Honey samples were gathered from eight different districts of Nepal, which were assessed for their color, ash content, moisture, electrical conductivity, pH, acidity, formaldehyde content, protein concentration, reducing sugar, total reducing sugar, sucrose content, and antibacterial properties. The color and protein were determined using a colorimeter, ash content using a muffle furnace at 550°C, moisture using a refractometer, and electrical conductivity using a conductivity meter. Acidity, formaldehyde, reducing sugar, total reducing sugar, and sucrose content were determined by the titrimetric method while the antibacterial potential was determined by the agar well diffusion method. The results indicated that six honey samples were dark amber and others were light amber in color. The mean values were found ranging from 18.4±0 to 20.8±0.69% for moisture, 0.05±0.04 to 1.35±0.26% for ash, 0.03±0.01 to 1184±5.26 µs/cm for electrical conductivity, 4.5±0.06 to 7.47 ±0.06 for pH, 90 to 137 meq/kg for acidity, 1 to 10 ml/kg for formaldehyde content, 0.28 to 0.73 mg/ml for protein concentration, 51.54% to 69.44% for reducing sugar, 65.35 to 80% for total reducing sugar, and 1.31 to 13.52% for sucrose. Out of the eight honey samples, four were able to show antimicrobial activity against *Staphylococcus aureus* ATCC 6538 while honey from Sindhupalchowk and Baitadi was also active against *S. epidermidis* ATCC 12228, and no other samples showed inhibitory activity against *Escherichia coli* ATCC 8739 and *Listeria monocytogenes* ATCC 19115. The honey sample from Lamjung was able to exhibit the highest antimicrobial activity against *Pseudomonas aeruginosa* ATCC 9027 at 1250 mg/ml concentration, while the lowest antimicrobial activity was shown by the sample from Chitwan at 750 mg/ml concentration against *S. aureus,* thus reflecting the significance of using honey as a natural remedy. This study indicated the essence of quantitative assessment of honey samples in terms of physicochemical parameters to ensure the quality of honey samples.

Keywords: Physicochemical properties, antibacterial activity, *S. aureus*, *P. aeruginosa*

Introduction

The use of different natural substances like honey has long been popular as an alternative for medicinal and remedial uses due to their nutritional value and effectiveness since the old medieval period to the present era (Meo et al., 2017). Honey is a saccharine and viscous food product made by the honeybee through extraction of nectar from blooms or exudate from any part of a plant and storing the finished product in honeycombs to mature and ripen (DFTQC, 2018 and CAC, 2001). Glucose and fructose monosaccharides make up the major constituents of honey with water and a number of minor elements such as polyphenols, minerals, volatile compounds, etc. (Hossain et al., 2020). The aroma, taste, and color of honey are influenced by different types of liquid present in the flowers and plants that honeybees visit (Meo et al., 2017).

Nepal produces a vast variety of honey and bee products due to its diverse climate and vegetation. Nepal has five different kinds of honeybees in different altitude regions, namely, the Asian hive bee (*Apis cerana*), European honeybee (*Apis mellifera*), giant honeybee (*Apis dorsata*), dwarf honeybee (*Apis florea*), and Himalayan cliff bee (*Apis laboriosa*). Most of the honey that is available comes from different kinds of flowers, plants, and fruits like mustard, sunflower, litchi, chiuri (an Indian butter tree), buckwheat, rudilo

(*Pogostemon* species), etc. (Devkota, 2020). It is estimated that Nepal has the ability to support one million beehives and can generate over ten hundred thousand metric tons of honey annually (Gautam et al., 2019). However, Nepali honey is unable to reach the full extent of its potential in the global honey market due to lack of quality certification (Bhattarai et al., 2019).

Honey has been acknowledged for its antibacterial and antioxidant properties, such as the ability to stop coughs, support reproduction, and capacity to heal lesions. It has been stated that inhibine (flavonoids, phenolic acids, and hydrogen peroxide) found inside honey is primarily responsible for its antibacterial activities that causes bacterial cell wall shrinkage and decrease in cellular pH. Similarly, alcohol produced from honey fermentation also has an inhibitory effect against bacterial growth. Irish et al. (2011) has reported that consumption of locally produced unadulterated honey limits the growth of microbes that leads to intestinal illnesses like *S.* Typhi and *E. coli.* Honey being one of the potent antibacterial agents available in nature has been reviewed for a number of conventional treatments. However, the potency of honey varies with different sources, concentrations, physicochemical properties, and bioactive components of honey (Albaridi, 2019). Thus, this work focuses on identifying physical and chemical prerequisites that could potentially be used to evaluate the reliability of diverse honey sources and to carry out antimicrobial activities against strong microbial infections with honey's potential inferences.

Materials and methods

Study site

The samples were collected from different locations of Nepal ranging from high altitudes (3000 m) to low lands (415 m). Eight honey samples from different districts of Nepal like Baitadi (BH), Chitwan (CH), Dhading (DH), Humla (HH), Lamjung (LH), Makwanpur (MH), Ramechhap (RH), and Sindhupalchok (SH) were collected.

Study duration

The study was carried out in the Microbiology Laboratory of St. Xavier's College, Maitighar, Kathmandu from December 2022 to June 2023.

Physical properties

Determination of pH

pH was determined according to the method described by Sereia et al. (2017). The pH was measured using a pH meter (HANNA Instruments Inc., USA) in a solution containing 10gm of honey in 75 ml of distilled water.

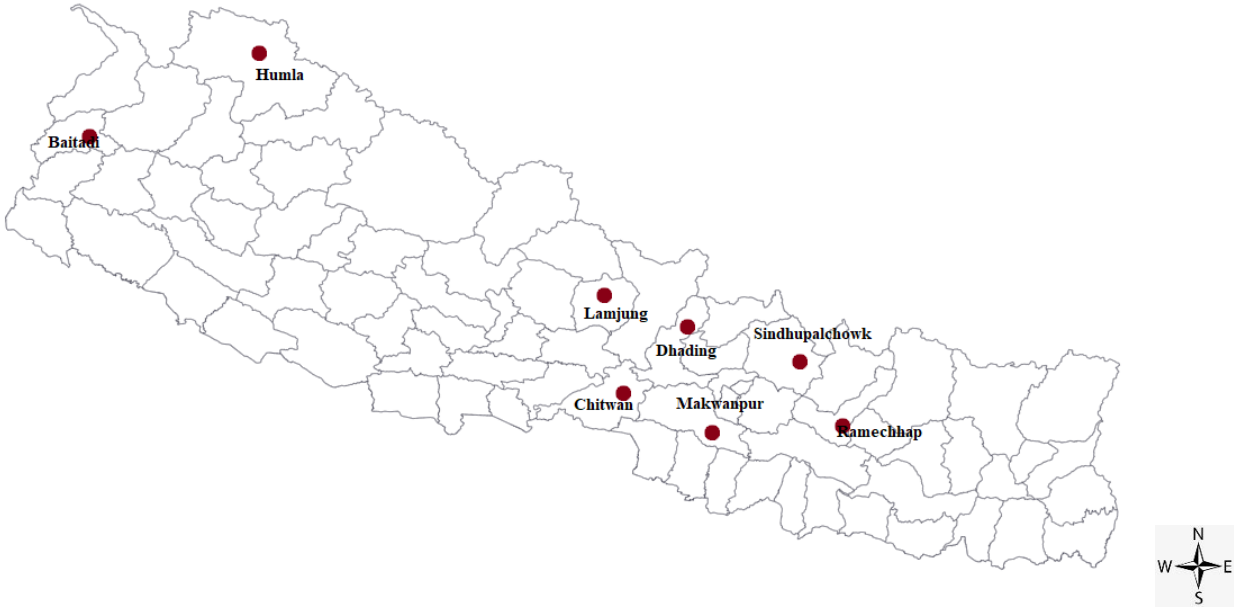


Figure 1. Locations of sample collection. (Source: Journal of Sustainable Forestry)

Determination of ash content

Approximately 3gm of the honey sample was charred by placing the crucible on the Bunsen burner until completely carbonized. The crucible was then placed in the muffle furnace at 550°C for 5 hours. After cooling to room temperature, the obtained ash was weighed for the measurement of the total ash content (Sereia et al., 2017).

Total ash content was calculated using the formula:

$$\text{Ash (\%)} = \frac{m_1 - m_2}{m_3} \times 100$$

Where $m_{1=}$ weight of crucible with ashes; $m_{2=}$ weigh of crucible; $m_{3=}$ weight of the sample

Determination of electrical conductivity

A conductivity meter (HANNA Instruments Inc., USA) was used to measure the conductivity from a solution containing 10 gm of honey in 50 ml distilled water. Prior to measuring the EC of the samples, the instrument was calibrated with 1412 µ*S*/cm buffer and waited till the reading stabilized (Sereia et al., 2017).

Determination of moisture content

The moisture content of the honey sample was determined using a refractometer (ERMA Inc., Japan). The refractometer was calibrated using distilled water and sugar solution. Three drops of the honey solution were placed in the refractometer, and after adjusting the angle limit, the reading of the refractive index was taken from the scale. The moisture content was correlated from the refractive index and water content correlation standard table.

Determination of color

The color of the honey sample was determined using a spectrophotometer (Thermo Fisher Scientific, USA) at wavelength of 560nm. The value was taken directly from the device, and a Pfund scale was used to determine the color according to the standard range (Sereia et al., 2017).

Chemical properties

Determination of acidity

Acidity was determined by the titrimetric method of Sereia et al., (2017). About 10gm of honey sample was mixed thoroughly in 75ml of distilled water, and 5 drops of phenolphthalein were added as an indicator. The solution was then titrated with 0.1N NaOH solution to pH 8.5. Then again 0.1N NaOH was added till the pH reached 10. The sample was then acidified by adding 0.1N HCl till the pH reached 8.3. The volumes of titrant consumed in each of the steps were noted to determine the acidity of the sample.

Free Acidity= corrected volume of NaOH × 10

Lactonic Acidity= (10 - corrected volume of HCl spent) ×10

Total acidity: free acidity- lactonic acidity

HCl corrected= volume of HCl spent × correction factor (fc)

NaOH corrected= volume of NaOH spent × correction factor (fc)

Determination of formaldehyde content:

The formaldehyde content was assessed just after determin ing the acidity by the method described in Sereia et al. (2017). When the pH of the sample reached 8.3, it was reduced to 8.0 by addition of 0.1N acetic acid followed by 5ml of 35% formalin. Then the solution was titrated against 0.1N NaOH till the pH returns to 8.0, and the total volume of the titrant consumed was noted. The formaldehyde content was measured using the formula :

Formaldehyde = Corrected volume of NaOH 0.1N spent×10 (mL kg^{-1})

Reducing sugar, total reducing sugar, and sucrose content

Reducing sugar, total reducing sugar, and sucrose content of honey was determined in accordance with the method described in Sereia et al., (2017).

For the titration of reducing sugar, the burette was filled with the reducing sugar solution. 5ml of Fehling A and Fehling B solution was added to a 250ml conical flask with 3-4 glass beads and 40ml of distilled water. The solution was then boiled and titrated against the reducing sugar solution. Once the color of the solution changed to purple, 5 drops of methylene blue indicator were added and boiled. The solution was again titrated against the reducing

sugar solution till the color of the solution changed to red earth color. The amount of titrant consumed was noted.

For the titration of total reducing sugar, the same procedure for the reducing sugar was followed but by using a total reducing sugar solution. The volume of titrant consumed was noted and calculated using the formula:

$$\text{Sucrose (\%)} = \frac{100 \times 100 \times 0.05}{0.5 \times V}$$

Where, V=Volume spent on titration, 0.05 = Correction factor for Fehling solution A & B

Saccharose (%) = (RS-TRS) ×0.95

Where, 0.95=Reducing factor from total reducing sugar, RS= reducing sugar, TRS= total reducing sugar

Protein

The Biuret method was used to determine the protein present in the honey sample. 5mg/ml of Bovine serum albumin (BSA) was prepared, and 0.2, 0.4, 0.6, 0.8 and 1ml of BSA was pipetted out into the series of labeled test tubes. A 1ml volume was made by adding distilled water in all the test tubes. A tube with 1ml of distilled water only served as a blank. About 1ml of sample solution and 3ml of biuret reagent were added to all the test tubes. The solution of the tubes was then vortexed and heated on a water bath at 37ºC for 10 minutes. The tubes were allowed to cool, and the absorbance was measured using a spectrophotometer. Then the standard curve was plotted by taking the concentration of protein along x-axis and absorbance at 540 nm along the y-axis. The standard curve was then used to determine the protein concentration of the sample (Plummer, 1988).

Antibacterial susceptibility test

The agar well diffusion method, as mentioned by Shrestha and Kandel, (2020), was followed with some modifications to evaluate the antibacterial properties. The antibacterial activity of the different concentrations of honey against five standard ATCC cultures (*Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, and *Staphylococcus epidermidis* ATCC 12228) was performed. These test organisms were tested for their resistance to antibiotics using the disc diffusion method as described by Bauer et al., (1966).

Honey samples were prepared using dimethyl sulphur oxide (DMSO) with concentrations of 1250 mg/ml, 1000mg/ml, 750mg/ml, 500mg/ml, and 250mg/ml. About 50 µl of honey with different concentrations were dispensed into five wells with the help of a sterile micropipette, while DMSO was used as the negative control. The plates were incubated at 37ºC for 24 hours. The mean diameters of inhibition zones were measured and noted.

Results

Physicochemical properties

Color, moisture content, ash content, electrical conductivity, and pH were some of the major physical properties to determine the quality of honey samples (Table 1). Of the eight honey samples analyzed, six were dark amber, and the others were classified as light amber ranging from 54.148±0 to 293.08±2.15 mm Pfund. While determining the moisture, it was observed that the honey with the highest moisture value was that of Makwanpur (20.8%) and with the lowest moisture value was that of Dhading (16.06%). The ash content values in the analyzed samples varied from 0.05±0.04% to 1.35±0.26%. The sample LH possessed the highest ash concentration, whereas sample SH showed the lowest ash content. The highest electrical conductivity was shown by sample LH with 1184 ± 5.29 µs/cm while the lowest electrical conductivity was shown by sample BH with a value of 0.03 ± 0.01 µs/cm. The pH values were found to be in the range of 4.5±0.06 to 7.47±0.06.

Chemical properties

Reducing sugar, total reducing sugar, and sucrose content of honey samples were determined using the titrimetric method. The reducing sugar content of the honey specimen ranged from 51.54% to 73.52%. Similarly, the total reducing sugar was observed at around 65.35% to 80%. Likewise, the sucrose content of the honey sample was found to be in the range of 1.31% to 11.40% (Table 2). In addition, free acidity of the honey samples during the study

was found to be in the range of 2 meq/kg to 54 meq/kg. Similarly, lactone acidity was in the range of 68 meq/kg to 92 meq/kg, and total acidity was found to be in the range of 90 to 137 meq/kg.

The formaldehyde content of the analyzed honey samples was found to be in the range of 1 to 10 ml/kg. The highest concentration of protein was recorded in sample DH with 0.73 ± 0.03 mg/ml while the lowest concentration of protein was estimated in sample HH with 0.28 ± 0 mg/ml (Table 2).

Antimicrobial properties

The honey samples were analyzed for their antibacterial activity against five ATCC cultures using the agar well diffusion method. The zone diameter of inhibition ranged from 9 to 16mm at 250 to 1250 mg/ml concentration of different honey samples (Table 3). Among all the honey samples taken, majority of the honey displayed antibacterial activity against *S. aureus* followed by *S. epidermidis*. In addition, honey sample from Lamjung showed the highest zone of inhibition against *P. aeruginosa* (Photograph 2, 3 & 4).

Table 1. Physical properties of honey samples

Sample	Color (Pfund)	Moisture (%)	Ash (%)	Electrical conductivity (µs/cm)	pH
DH	Dark amber (263.37±2.14)	16.06±0.57	0.35±0.50	836.33±5.50	5.23±0.15
HH	Light amber (78.90±2.14)	19.73±0.57	0.08±0.08	358.33±2.51	4.70±0.10
SH	Dark amber (181.66±2.14)	18.4±0	0.05±0.04	176.4±1.75	5.73±0.11
RH	Dark amber (208.89±2.15)	19.4±0	0.22±0.06	712±3.60	4.73±0.05
LH	Dark amber (293.08±2.15)	19.06±0.57	1.35±0.26	1184±5.29	7.46±0.05
BH	Light amber (54.148±0)	19.4±0	0.12±0.08	0.03±0.01	4.50±0.05
MH	Dark amber (142.04±2.14)	20.8±0.69	0.44±0.25	969.66±5.50	5.43±0.11
CH	Dark amber (175.47±2.15)	18.4±0	0.28±0.07	903±7.54	5.93±0.23

where, DH -Dhading, HH- Humla, SH- Sindhupalchok, RH- Ramechhap, LH- Lamjung, BH-Baitadi, MH-Makwanpur, CH- Chitwan.
Values are mean values of three replicate samples (n=3)

Table 2. Chemical properties of honey samples

Sample	RS (%)	TRS (%)	S (%)	FA (meq/kg)	LA (meq/kg)	TA (meq/kg)	F (ml/kg)	P (mg/ml)
DH	69.44%	77.51%	7.66%	22	89	111	4	0.73 ±0.03
HH	65.35%	71.94%	6.26%	32	92	124	6	0.28 ± 0
SH	68.96%	80%	10.48%	20	78	98	1	0.63 ± 0.05
RH	73.52%	80%	6.15%	54	83	137	8	0.63 ± 0
LH	55.55%	67.56%	11.40%	2	88	90	7	0.28 ± 0.03
BH	67.11%	68.49%	1.31%	46	68	114	10	0.63 ± 0
MH	60.24%	65.35%	4.85%	13	87	100	3	0.61 ± 0.03
CH	51.54%	65.78%	13.52%	9	91	100	3	0.41 ± 0.03

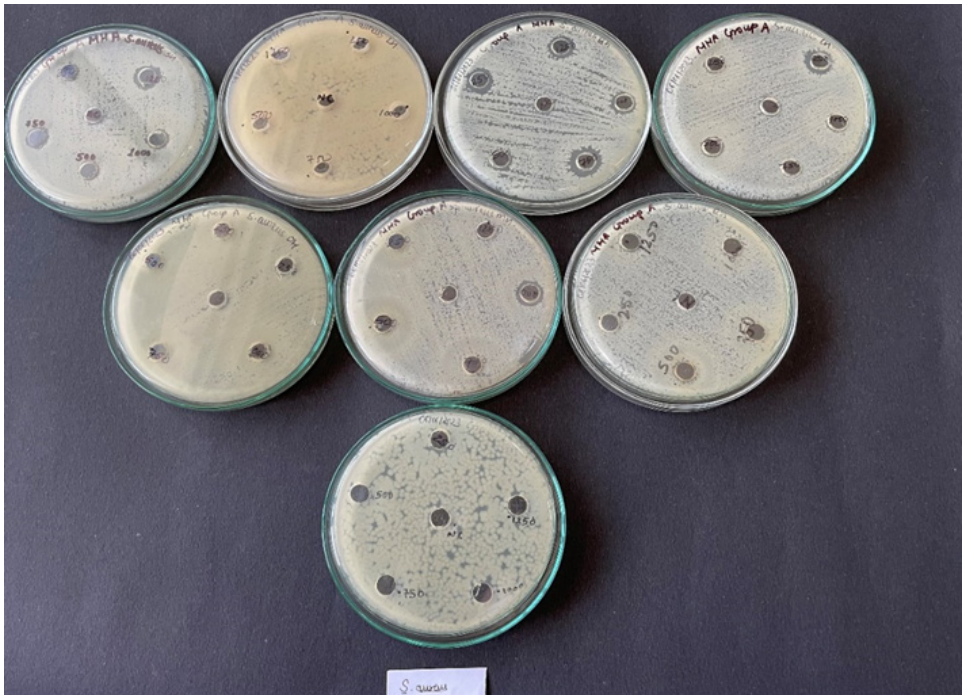
Where, RS=Reducing sugar, TRS= Total reducing sugar, S= Sucrose, FA= Free acidity, LA= Lactonic acidity, F= Formaldehyde, P=Protein, DH -Dhading, HH- Humla, SH- Sindhupalchok, RH- Ramechhap, LH- Lamjung, BH-Baitadi, MH-Makwanpur, CH-Chitwan. Values are mean values of three replicate samples (n=3)

Table 3. Antimicrobial susceptibility status of different honey samples

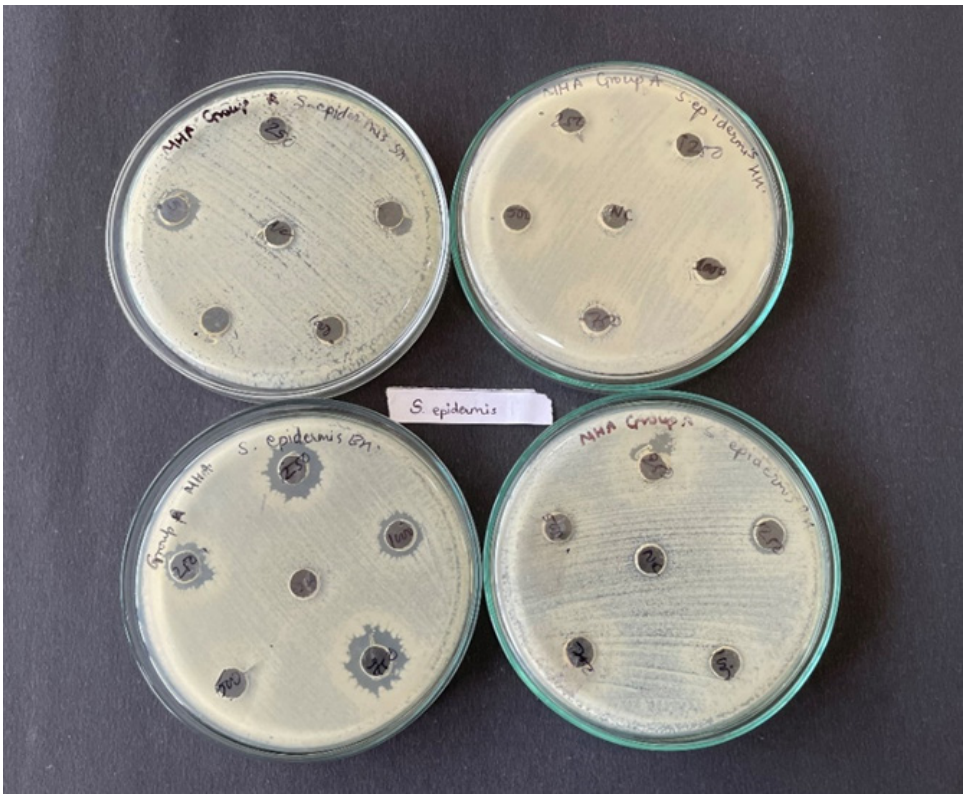
Sample	Test organism	Concentration (mg/ml)				
		1250	1000	750	500	250
DH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	0	0	0	0	0
	S. epidermidis	0	0	0	0	0
HH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	0	0	0	0	0
	S. epidermidis	0	0	0	0	0
SH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	13mm	11mm	11mm	0	0
	S. epidermidis	13mm	0	0	0	0
RH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	0	0	0	0	0
	S. epidermidis	0	0	0	0	0
LH	*E. coli*	0	0	0	0	0
	L. monocytogenes		0	0	0	0
	P. aeruginosa	16mm	13mm	12mm	12mm	11mm
	S. aureus	0	0	0	0	0
	S. epidermidis	0	0	0	0	0
BH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	15mm	14mm	12mm	11mm	11mm
	S. epidermidis	12mm	0	0	0	0
MH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	13mm	0	0	0	0
	S. epidermidis	0	0	0	0	0
CH	*E. coli*	0	0	0	0	0
	L. monocytogenes	0	0	0	0	0
	P. aeruginosa	0	0	0	0	0
	S. aureus	13mm	11mm	9mm	0	0
	S. epidermidis	0	0	0	0	0

where, DH -Dhading, HH- Humla, SH- Sindhupalchok, RH- Ramechhap, LH- Lamjung, BH-Baitadi, MH-Makwanpur, CH- Chitwan

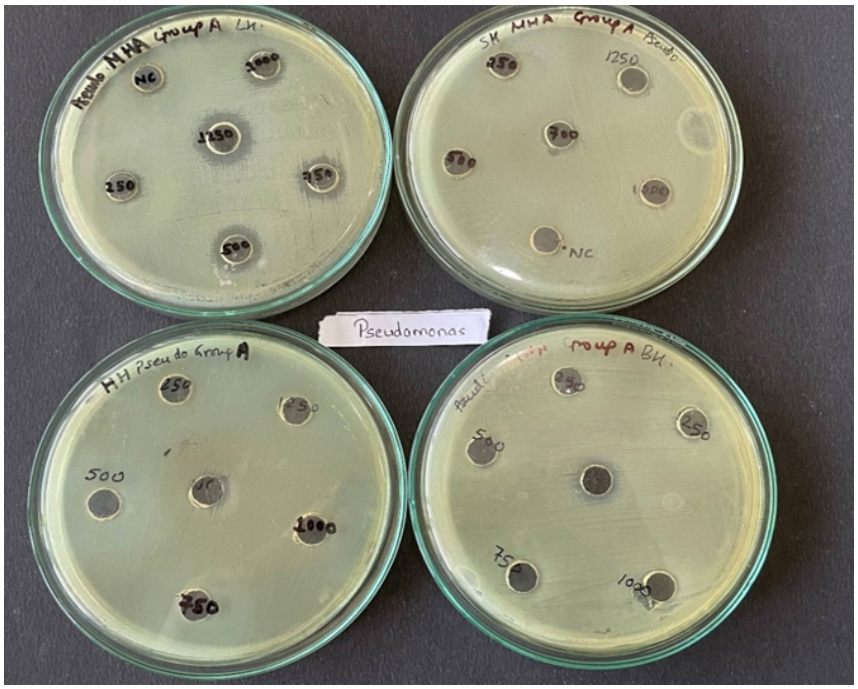
Photograph 1. Honey samples collected from various districts of Nepal (DH -Dhading, HH- Humla, SH- Sindhupalchok, RH- Ramechhap, LH- Lamjung, BH-Baitadi, MH-Makwanpur, CH- Chitwan)



Photograph 2. Honey samples showing antibacterial activity against *S. aureus* ATCC 6538 at concentration of 1250 mg/ml, 1000 mg/ml, 750 mg/ml, 500 mg/ml, and 250mg/ml



Photograph 3. Honey samples showing antibacterial activity against *S. epidermidis* ATCC 12228 at concentration of 1250 mg/ml, 1000 mg/ml, 750 mg/ml, 500 mg/ml, and 250 mg/ml



Photograph 4. Honey samples showing antibacterial activity against *P. aeruginosa* ATCC 9027 at concentration of 1250 mg/ml, 1000mg/ml, 750 mg/ml, 500 mg/ml, and 250 mg/ml

Discussion

In this study, eight honey samples were collected from different parts of Nepal at altitudes ranging from 415m-3000m. The honey samples were focused on studying their physicochemical and antimicrobial activity. During this study, honey samples mostly exhibited dark amber and light amber color (Photograph 1). The color of the honey could vary depending on its floral source, mineral content, storage and processing methods, environmental conditions during nectar flow, and the temperature at which the honey grows in the hive. According to Royo et al. (2022), dark honey is rich in minerals, have higher concentrations of calcium, iron, vitamins B and C, and with a stronger aroma. Aazza et al., (2018), Sajid et al. (2020), and Boussaid et al. (2018) reported the color range between 1.03-71.27 mm Pfund, 27.95-78 mm Pfund, and 36.64-51.37 mm Pfund, .showing significant variations with this study.

It was observed that the moisture content of the honey sample from Makwanpur was the highest (20.8%) as shown in Table 1, indicating that it could have been harvested prematurely, and there is a possibility that the honey might undergo fermentation and form acetic acid. The sample obtained from Dhading exhibited the lowest moisture value (16.06%) indicating that there is less probability of fermentation by osmophilic microorganisms due to low water activity. In the present study, the moisture contents of the examined honey samples were found to be in the range of 16.06 ± 0.57% to 20.8 ± 0.69%, which were between the permitted limits of 21% according to CAC, (2001). The values of our study were similar to those previously reported for different kinds of honey whose corresponding values ranged from 19.30 ± 0.87% to 20.15 ± 1.39 % (Bhattarai et al., 2019).

The ash content indicates the inorganic content, which points to the floral source of honey and distinguishes between nectar honey and honeydew honey. It also serves as a measure of the mineral content. The sample from Lamjung possessed the highest ash concentration (1.35%) exceeding the standard set by the CAC (≤ 0.6 g/kg). The ash percentage reported by Parviz et al., (2015) of Iranian honey was between 0.17 and 0.8%. The variation in soil and physiological variations in the plants from different geographical locations might have been the causes for the differences in the ash content.

The ash content is directly correlated to the mineral contents of the honey sample. Hence, honey from Lamjung having higher ash showed high electrical conductivity. The values for EC of the honey samples (SH, RH, BH, and CH) were within the standard limit (≤800 µS/cm) approved by the CAC. Joshi et al. (2000) determined the electrical conductivity of honey samples from Chitwan to be in the range of 0.31 to 0.96 mS/cm.

The acidic pH increases the quality and shelf life of honey. The pH values of the honey samples used in this study were found to be within the standard limit of pH 3.40 to 6.10 approved by the CAC except for the LH sample. Honey samples with a pH above 5 is considered to be of low quality as there is possibility of microbial growth because of the presence of various organic acids like pyruvic acid, citric acid, and gluconic acid. Joshi et al. (2000) reported that the pH values range from 3.52 to 3.68, which was higher than our finding. The acidity of honey results from the organic and inorganic acid present in it as well as gluconic acid produced by the action of glucose oxidase produced in the hypopharyngeal glands of the bees (Sereia et al., 2017).

Sample RH exhibited the greatest reducing and total reducing sugar concentration while the lowest reducing and total reducing sugar concentration were shown by sample CH and MH, respectively. The sucrose content of CH, DH, HH, LH, RH, and SH was higher than the Nepal standard of maximum 5% (DFTQC, 2018). High amounts of sucrose indicated the early harvest of honey. Bhattarai et al., (2019) had reported the reducing sugar content of honey from different floral sources to be in the range of 64.06 ± 1.99% to 70.76 ± 1.26%, which was lower than our findings. In the context of total reducing sugar, Bhattarai et al., (2019) had reported the values to rang e from 70.38 ± 2.21% to 75.22 ± 0.38%, which was comparably higher in terms of our findings. Bhattarai et al., (2019) reported similar observations in the case of sucrose content, where the data was found to be at around 4.71 ± 0.89%

to 7.24 ± 0.77%, which was lower than our results, respectively.

In this study, free acidity of the honey samples was found to be in the range of 2 meq/kg to 54 meq/kg (Table 2). This exceeded the maximum limit specified as satisfactory in international trade (50 meq/kg). Free acidity and lactonic acidity are considered as the acidity reserve when the honey becomes alkaline. Highest lactonic acidity was observed in HH (92 meq/kg) while the lowest was BH (68 meq/kg). The highest total acidity was also observed in BH (144 meq/kg) and the lowest in SH (98 meq/kg). All the samples were above the acceptable limit of less than 50 meq/kg (CAC, 2001). This concluded that there is presence of undesirable fermentation. The acidity of honey results from the organic and inorganic acid present in it as well as gluconic acid produced by the action of glucose oxidase produced in the hypopharyngeal glands of bees (Sereia et al., 2017). Similar results were obtained in the study conducted by Bhattarai et al. (2019), which suggested that variation in the results might have occurred due to the presence of fermentation or seasonal change for the sources of the flowers being used for the honey samples.

Formaldehyde in honey indicates the presence of nitrogen in honey. The highest formaldehyde content was observed in the BH sample. The lowest value was observed in SH, indicating that there may be the presence of artificial products. According to Sereia et al. (2017), if present in excessive amounts, it demonstrates that the bees might have been fed with hydrolyzed protein. Formaldehyde should be present in an undetectable amount because its existence indicates that honey has been adulterated.

The highest concentration of protein was recorded in the sample obtained from Dhading with 0.73 ± 0.03 mg/ml while the lowest concentration was estimated in sample HH with 0.28 ± 0 mg/ml (Table 2). Proteins found in honey originate from the honey bees rather than from the nectar and are in the form of enzymes. Sohaimy et al., (2015) reported that the highest protein level was found in Kashmiri honey (4.67 ± 0.171 mg/g), which was higher than our findings. It has been speculated that the protein level of honey varies based on its origin and types of pollen present.

Among all the honey samples taken, majority of the honey displayed antibacterial activity against *S. aureus* followed by *S. epidermidis* (Table 3), emphasizing the fact that honey is able to show antimicrobial activity against Gram-positive organisms. In a similar study conducted by Shrestha and Kandel (2020), antibacterial activity of honey samples was found effective against isolates of *S. aureus*. According to Hossain, et al. (2020), antibacterial action could be regulated by a variety of qualities, such as high sugar concentration, low water content, acidity, hydrogen peroxide, and non-peroxide content, etc. In addition, the honey sample from Lamjung showed a higher zone of inhibition against *P. aeruginosa*. In a study conducted by Jenkins et al. (2011), it was demonstrated that a variety of bacteria such as *P. aeruginosa* and *E. coli* were highly susceptible to raw honey.

This study highlighted the varied physicochemical and antibacterial properties of honey samples from different geographical locations. However, the evaluation of antioxidant properties along with minimum inhibitory concentration and minimum bactericidal concentration would have better defined their potential as a natural antioxidant and antimicrobial agent.

Conclusion

The study revealed that the significant difference in the characteristics of honey is due to the varied geographical origin of honey. Majority of the analyzed Nepali honey samples were of suitable quality in accordance with international standards. Most of the honey samples showed antimicrobial activity against *S. aureus*. The variation in values seems to be the result of changes in environmental factors, particularly due to varied climatic conditions, floral source, and storage conditions.

With reference to their physicochemical parameters and antimicrobial activity, this study can assist in making better quality honey products. These factors are essential not only for quality control and certification but also to serve as benchmarks for international trade. The experiment demonstrated the purpose of selecting honey not just as a nutritional

food source but also as possible antimicrobials against diseases caused by microorganisms. According to our research, choosing honey based on botanical origin may be necessary for the effective analysis of physicochemical, antimicrobial, and other properties to explore the various essential components required for the development of medicines.

Acknowledgement

We would like to express our sincere gratitude to Ms. Pramila Parajuli, Head of the Department, Microbiology for their support and encouragement. Special thanks to all the Microbiology laboratory staff for their kind cooperation and providing the necessary materials for the study.

References

Aazza, S., Elamine, Y., El-Guendouz, S., Lyoussi, B., Antunes, M. D., Estevinho, L. M., Anjos, O., Carlier, J. D., Costa, M. C., & Miguel, M. G. (2018). Physicochemical characterization and antioxidant activity of honey with Eragrostis spp. pollen predominance. *J. Food Biochem.*, 42(1), e12431. https://doi.org/10.1111/jfbc.12431

Albaridi, N. A. (2019), Antibacterial Potency of Honey, *Int. J. Microbiol.*, *2019*, 1–10. https://doi.org/10.1155/2019/2464507

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clini. Pathol.*, *45*(4_ts), 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bhattarai, S., Subedi, U., Kumar Bhattarai, U., Karki, R., & Ojha, P. (2019), Study on Chemical and Bioactive Components of Different Floral Sources Honey in Nepal, *J. Food Sci. Technol. Nepal*, 11, 51–59.

Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsì, F., Ferrari, G., & Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arb. J. Chem*., 11(2), 265–274. https://doi.org/10.1016/j.arabjc.2014.08.011

CAC. (2001). The Revised Codex Standards for Honey, *Codex Alimentarius Commission Standards CODEX STAN*, *12–1981*, Rev.1 1987, Rev.2 2001 1-8. Retrieved from https://www.fao.org/3/w0076e/w0076e30.htm, Accessed on (15/02/2023)

Devkota, K. (2020), Beekeeping: Sustainable Livelihoods and Agriculture Production in Nepal, *Modern Beekeeping - Bases for Sustainable Production.* IntechOpen. 11, 10-13. https://doi.org/10.5772/intechopen.90707

DFTQC. (2018), Minimum mandatory standard for food and feed products. *Department of Food Technology and Quality Control, Ministry of Agriculture and Livestock Development, Government of Nepal*, *50*(3), 1–156. Retrieved from: https://ftqcdojumla.dftqc.gov.np/wp-content/uploads/2021/06/अनिवार्य-गुणस्तर-२०७५.pdf, Retrieved Feb 15, 2023

Hossain, K. S., Hossain, M. G., Moni, A., Rahman, M. M., Rahman, U. H., Alam, M., Kundu, S., Rahman, M. M., Hannan, M. A., & Uddin, M. J. (2020), Prospects of honey in fighting against COVID-19: pharmacological insights and therapeutic promises, *Heliyon*, 6(12), e05798. https://doi.org/10.1016/j.heliyon.2020.e05798

Irish, J., Blair, S., & Carter, D. A. (2011), The antibacterial activity of honey derived from Australian flora, *PLoS One*, 6(3). https://doi.org/10.1371/journal.pone.0018229

Jenkins, R., Burton, N., & Cooper, R. (2011), Manuka Honey Inhibits Cell Division in Methicillin-Resistant *Staphylococcus aureus*, J. Antimicrob. Chemother., 66(11), 2536–2542. https://doi.org/10.1093/jac/dkr340

Joshi, S. R., Pechhacker, H., Willam, A., & Ohe, W. Von der. (2000). Physico-chemical characteristics of *Apis dorsata*, *A. cerana* and *A. mellifera* honey from Chitwan district, central Nepal. 367–375.

Meo, S. A., Al-Asiri, S. A., Mahesar, A. L., &

Ansari, M. J. (2017). Role of honey in modern medicine. *Saudi. J. Biol. Sci*., 24(5), 975–978. https://doi.org/10.1016/j.sjbs.2016.12.01012.010

Plummer D.T., (1988) *An Introduction Practical Biochemistry*, Tata Mc Graw-Hill Publishing Company, New Delhi. p.159.

Parviz, M., Karimi, F., Rezaei, M., Javanmard, M. R., Javadzadeh, M., & Allahdadi, G. (2015). Assessment of the physicochemical quality of Iranian honey. *Quality assurance and safety of crops & foods*, *7*(5), 629-634.

Sajid, M., Yamin, M., Asad, F., Yaqub, S., Ahmad, S., Mubarik, M. A. M. S., Ahmad, B., Ahmad, W., & Qamer, S. (2020). Comparative study of physio-chemical analysis of fresh and branded honeys from Pakistan. *Saudi. J. Biol. Sci*., 27(1), 173–176. https://doi.org/10.1016/j.sjbs.2019.06.014

Sereia, M. J., Março, P. H., Perdoncini, M. R. G., Parpinelli, R. S., de Lima, E. G., & Anjo, F. A. (2017). Techniques for the Evaluation of Physicochemical quality and bioactive compounds in honey. In *Honey Analysis* (193–214). InTech. Retrieved May 15, 2023. https://doi.org/10.5772/66839

Shrestha, A., & Kandel, M. (2020), "Antibacterial activity of honey on *Staphylococcus aureus* isolated from wounds," *NJST*, 19(1), 167–170. https://doi.org/10.3126/njst.v19i1.29797

Sohaimy E., S. A., Masry, S. H. D., & Shehata, M. G. (2015). Physicochemical characteristics of honey from different origins. *Ann. Agri. Sci.*, 60(2), 279–287. https://doi.org/10.1016/j.aoas.2015.10.015