Biological Activity Level (BAL) of Honey

Honey has been used as a medicine by every civilization on earth due to its medical properties and biological activities originating from its nectar source. The honeybees forage the flower blossoms, tree secretions, and insect excretions to collect the nectar and convert it to honey using the enzymes within their abdomens. Since these nectars provide various chemical constituents such as phenolic acids, flavonoids, tannins, pigments, minerals, and vitamins; every honey's biological activity differs from each other based on the abundance and ratios of these compounds. And these components vary in accordance with the terroir, climate, foraging window, and honeybee strain. Therefore, we can conclude that every honey is created differently.

The surge in honey's use in modern medicine places an unprecedented emphasis on the need for medically safe and effective honey. According to most recent studies, Medical-Grade Honey (MGH) must be produced in organic environments free from pesticides, meet rigorous harvesting, production, and storage standards, undergo gamma sterilization, and adhere to clinical safety benchmarks. Above all, MGH must possess biological activities, which are identified using the physicochemical, antioxidant, and antimicrobial characteristics of the honey samples.

Dr. Ozturk's research explores the medicinal potential of Texan honey varieties, introducing a comprehensive Biological Activity Level (BAL) formula that amalgamates these properties. The study presents a novel approach that integrates both physical and chemical properties, along with the antibacterial and antioxidant potential, to gauge honey's biological activity.

An array of honey samples from diverse regions across Texas underwent various methodologies to compute the BAL for each sample. Based on their BAL values, the honey samples were classified as having notably high or low bioactivity levels. Notably, honey from the San Antonio-Hill Country region exhibited a particularly high BAL value. These findings underscore the remarkable medical-grade potential inherent in locally sourced Texas honey.

The honey analysis certificates contain the following measurements:

1. Physicochemical Properties

1.1. Color Detection.

The color of honey is strongly related to the botanical origin of the honey sample and can be used to assess the quality of the honey. According to the United States Department of Agriculture, there are 7 colors of honey and honey color standard designations are expressed using the Pfund scale.

Table 1 - Color Designations of Honey

USDA Color Standard Designation	Color Range Pfund Scale (mm)	Sample Result Range
Water White	≤8	0-0.094
Extra White	>8 and ≤17	0.094- 0.189
White	>17 and ≤34	0.189-0.378
Extra Light Amber	>34 and ≤50	0.378-0.595
Light Amber	>50 and ≤85	0.595-1.389
Amber	>85 and ≤114	1.389-3.008
Dark Amber	>114	>3.008

1.2 pH The pH range of honey to be classified as acidic is between 3.2 and 4.5. The acidic nature of honey contributes to antimicrobial activity because it is low enough to inhibit bacterial pathogens. While most honey creates an environment to inhibit bacterial growth, not all the

Texas honey samples analyzed were acidic. The acidity value of the samples ranged from 3.73 - 6.76, with an average of 4.94. The low pH can inhibit the growth of bacteria and is an indicator of the overall quality of honey. The pH of honey sample was evaluated using the Accumet pH meter (Fisherbrand). 40% of honey solutions were prepared using 2g of honey sample diluted in 5mL DI water in borosilicate glass tubes.

1.3 Moisture Content.

Honey is characterized by low moisture content and as a result, few bacteria can survive in this environment, therefore low water activity the greater antibacterial environment. The moisture content of the honey samples ranged between 14.40 and 22.00, with an average of 17.53% the honey samples are in range for what an acceptable moisture content should be which is typically around 17% - 18%. The amount of moisture affects various characteristics including the color, viscosity, flavor, density and more importantly, fermentation which affects the color of honey, changes its flavor, and alters its shelf life

2. Antimicrobial Properties

2.1 Zone of Inhibition.

The antibacterial activity of the honey samples was determined using the agar well-diffusion method on *Staphylococcus aureus* bacteria to measure the zone of inhibition after 18-h incubation at 37°C with the presence of honey samples. The zones higher than 16mm were considered as high antimicrobial activity according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

3. Antioxidant Properties

3.1 DPPH Radical Scavenging Assay (RSA).

The DPPH radical-scavenging assay (RSA) is based on the ability of antioxidants to block the free radical of DPPH (2,2-difenil-1-picril-hidrazil) and is used to measure the antioxidant activity of the honey samples. Due to its simplicity, DPPH assay has been the most used method to measure honey's antioxidant activity. The RSA of honey sample was determined as a percentage based on the discoloration of DPPH after 30-minute incubation. The higher percentage RSA, the higher antioxidant activity of the honey sample.

4. Biological Activity Level (BAL)

Percentage	BAL Value
>81%	Very High
61% to 80%	High
41% to 60%	Moderate
21% to 40%	Low
<20%	Very Low

BAL values of the TX honeys were calculated using a special formula including the three major properties of each honey sample and converted to percentages according to the highest BAL value among 193 samples.

Honeys were classified using the percentage table.