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Abu Dhabi Honey – A Good Source Of Hydrogen Peroxide And Antimicrobial Activity

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Abstract
Honey is a sugary viscous liquid having known for its therapeutic potential since ancient times. It is packed with nutrients and compounds that have antimicrobial, anti-inflammatory and anticancer properties. The hydrogen peroxide production resulting from the enzymatic action of glucose oxidase produced by the hypopharyngeal glands of honeybees has a linear correlation with antimicrobial property. In the current study, hydrogen peroxide content of Sidr and Damas honey produced in the Emirate of Abu Dhabi were assessed for 101 samples. The mean concentration of hydrogen peroxide in Sidr honey was 1.69 ± 0.79 mM, while it was 2.56 ± 0.84 mM in Damas honey. A statistically significant difference between Sidr and Damas honeys has been observed. Antimicrobial efficacy using the well diffusion method against Staphylococcus aureus exhibited inhibition zones ranging from 20 to 27 mm implying strong antibacterial activity. Further studies involving more samples and test organisms could assess the potential application in complementary medicine and food preservation.
Keywords: *honey; hydrogen peroxide; antimicrobial, Sidr; Damas;*

Background

Honey is a sugary viscous liquid having known for its therapeutic potential since ancient times. It contains a unique blend of organic and inorganic compounds predominantly influenced by botanical and geographical sources (1). It is revered as sacred food in many religious practices including Hinduism, Christianity and Islam (2). According to one of the oldest sacred text of Hindus, the rig Veda (4000 BCE-1500 BCE), honey is described as sweet and healing food and mentions about the role of bees in honey production (3). In the case of Bible, honey appears prominent in Old Testament 'a land flowing with milk and honey' indicating vegetation across the land and conducive environment for milk production (4). In Islam, there exists a chapter dedicated to bees and Prophet Mohamed (PBUH) has mentioned it for healing purposes (5).

Honey has many key advantages to be considered as a product beyond food packed with nutrients and compounds having antimicrobial, anti-inflammatory, anticancer properties (6, 7, 8). For instance, the antimicrobial property of honey is influenced by the physiochemical properties such as low pH, high sugar content, low water activity, production of hydrogen peroxide and non-peroxide based bioactive compounds (6, 9, 10). With regards to hydrogen peroxide based antimicrobial activity, a linear correlation exists between the amount of hydrogen peroxide produced and the minimum inhibitory concentration (MIC) with a reduced activity upon treatment with catalase suggesting the key role of hydrogen peroxide in the antibacterial activity of honey (11). The Hydrogen peroxide production in honey is resulting from enzymatic action of glucose oxidase (GOx) produced by the hypopharyngeal glands of the honeybees (9). The enzyme catalyzes the oxidation of glucose to hydrogen peroxide and gluconic acid. However, recent studies suggest that it is a complex network of reactions involving several factors (12) which include colony health, diet, catalase obtained through

pollen etc. In Manuka honey produced in Australia and New Zealand, a non-peroxide based antimicrobial activity by methylglyoxal (MGO) is found in high concentrations exhibiting a broad-spectrum antimicrobial activity through chemical conversion of dihydroxyacetone (DHA) from the nectar of the manuka tree, *Leptospermum scoparium* (13).

As far as honey production in the Emirate of Abu Dhabi is concerned, it is a strategic program promoted by the local government as part of sustainable agricultural development to support food security. In this context, an Emirati bee breed exhibiting distinctive traits adapting to the local environmental conditions has also been raised successfully. The diverse desert floral blooms such as Sidr, (*Ziziphus spina-christi*), damas (*Conocarpus lancifolius*), qurm (*Rhizophora sp.*) and ghaf (*Prosopis cineraria*) provide a conducive environment for honey production reflecting the distinct flavors and aromas of the region having the possibility of unique physiochemical properties reflecting the floral and regional conditions.

With this background, the aim of the current study is to explore the hydrogen peroxide content of Sidr and Damas honey produced in the Emirate of Abu Dhabi and its antimicrobial properties.

MATERIALS AND METHODS:

Honey samples:

A total of 101 honey samples from 2 floral sources, namely Sidr and Damas were collected from the Emirate of Abu Dhabi. All samples were stored in glass containers at 25°C until analysis. The samples were homogenized by stirring thoroughly for at least 2 minutes before analysis.

Chemicals and Reagents

The following laboratory grade chemicals were obtained from Sigma-Aldrich. (St. Louis, MO, USA): O-Dianisidine, Horseradish peroxidase type II, Sulfuric acid (6M), Whatman filter paper (42), Ethanol, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Hydrogen peroxide and ultrapure water from an ultrapure water purification system with resistance of 18.2 $\mu\Omega$-cm was used throughout (Millipore, Bedford, MA).

Physicochemical and organoleptic properties of honey

All physicochemical analyses were performed in accordance with UAE standards. Briefly, samples were tested for Moisture content, Acidity, Electrical conductivity, Diastase activity, Hydroxymethylfurfural analysis, sugar composition, heavy metals, yeast and mold counts as described in UAE S 147: 2019.

Quantification of Hydrogen Peroxide

Quantification of hydrogen peroxide in the honey samples was determined enzymatically based on the reaction of hydrogen peroxide with *o*-dianisidine in the presence of horseradish peroxidase type II to form a colored product (14). A standard curve was constructed using a series of known concentrations of standard hydrogen peroxide solutions. The absorbance of the unknown honey sample was determined by comparing its absorbance to the standard curve.

Standard preparation and analysis

Five-point calibration involving hydrogen peroxide concentration ranging from 0.005, 0.01, 0.05, 0.1, 0.25mM standards solutions were prepared to construct a calibration curve.

In the case of samples, 30±0.1 grams of each honey sample was weighed using an analytical balance followed by the addition of 40 ml of buffer solution (pH 6.5) and mixed. After complete dissolution, the sample volume was made up to 100 ml by adding distilled water. The mixture was filtered through Whatman 42 filter paper twice to ensure clarity and remove any particulate matter. The filtered sample (30 ml) was mixed with 100 ml of peroxide reagent to let the reaction proceed for 10 minutes followed by the addition of 0.1 ml of 6M sulfuric acid. The optical density of the solution was

measured at 410 nm and the results were recorded. All measurements were performed in triplicate for each sample and the results were expressed as mM of hydrogen peroxide in honey solution.

Antimicrobial activity

To determine the antimicrobial activity, an inoculum concentration of 10^6 CFU per ml, was prepared and honey was diluted with sterile distilled water to desired concentrations (e.g., 25%, 50%, 75%, 100%v/v) and the dilution exhibiting maximum diffusion in the agar medium was selected. Mueller-Hinton Agar plates were inoculated with 100 µL of standardized microbial suspension using a sterile spreader evenly to create a lawn culture and kept for 10-15 minutes. Using a sterile cork borer, 6 mm wells were punched in the agar and filled each well with 100 µL of honey sample (test concentrations), controls, and reference antibiotics. The plates were incubated at 35-37°C for 18-24 h. After incubation, the diameter of zone of inhibition (in mm) around each well was measured using a caliper and the results were recorded in triplicate for each organism and condition.

RESULTS AND DISCUSSION

The therapeutic properties of honey obtained through different floral sources, especially hydrogen peroxide production through enzymatic reaction contribute significantly to antimicrobial activity having wider application in wound healing and in the prevention of dental plague and cavities (15,16).

A total of 101 honey samples (62 Sidr and 39 from Damas floral sources) have been utilized in the current study. All samples were complaint for physicochemical parameters conducted as per standard requirement. The box plot represents the concentration of all Sidr and Damas samples applied in this study (fig. 1). The mean concentration of hydrogen peroxide in Sidr honey was 1.69 ± 0.79 mM, with values ranging from 0.3 ppm to 4.584 mM. In contrast, Damas honey exhibited a significantly higher mean concentration of 2.56 ± 0.84 mM, ranging from 1.5 to 4.4 mM. The result of our study corroborates with some of the previous findings suggesting that Sidr honey generally contains moderate H_2O_2 levels (17,18). Nevertheless, concentrations above 1.5mM are generally considered sufficient to inhibit microbial growth due to honey's acidity and osmotic effect. In the case of Damas honey, peroxide concentration exceeds the standard antimicrobial levels for honeys used in wound care and infection control (19).

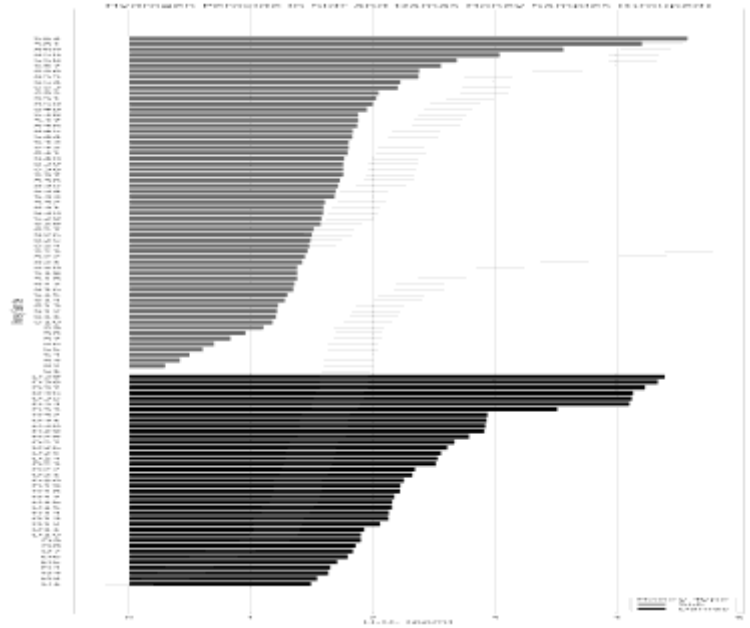

Fig. 1. Concentration of H2O2 in honey samples

The vast difference may be attributed to the levels of glucose oxidase and catalase enzymes in different floral sources. For instance, honey with low catalase levels exhibit high H2O2 concentration.

A two-sample independent t-test assuming unequal variances was performed to assess the statistical significance of the difference in hydrogen peroxide concentrations. The results indicated a statistically significant difference between Sidr and Damas honeys ($t = -5.19$, $p < 0.00001$), confirming that Damas honey contains substantially higher levels of hydrogen peroxide.

The relatively high standard deviation in both honey types indicates noticeable batch-to-batch variability. In Sidr honey, the hydrogen peroxide content spanned a wide range, with some samples close to or below the detection threshold. Such variability may reflect differences in nectar composition, harvest season, and storage. Previous studies have also reported similar fluctuations in H_2O_2 content in honeys depending on harvest year and storage practices (20).

To assess the actual antimicrobial efficacy, samples of Sidr and Damas honey were evaluated using the agar well diffusion method against *Staphylococcus aureus*. Sidr honey exhibited inhibition zones ranging from 20 to 22 mm (mean: 21.2 ± 0.84 mm), while Damas honey displayed larger zones between 25 and 29 mm (mean: 27.0 ± 1.73 mm).

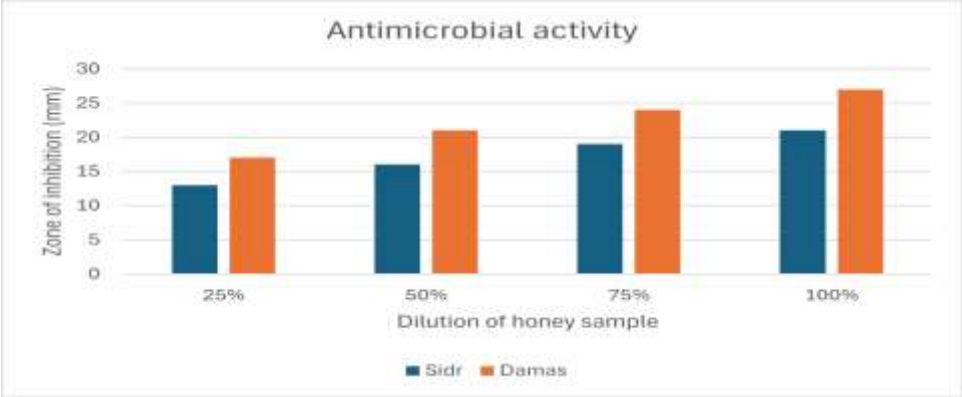

Fig. 2. Zone of inhibition for different dilution

Although both honey types possess significant antibacterial activity, Damas honey demonstrated a markedly higher efficacy. In general, inhibition zones greater than 18 mm imply strong antibacterial activity (21) and both honey types were well within this category. Neverthelss, the superior performance of Damas honey correlates to higher levels of hydrogen peroxide concentration (fig. 2). The statistical comparison confirms the difference between the mean zone of inhibition of Sidr and Damas honey ($p < 0.05$), indicating that botanical origin plays a pivotal role in the antimicrobial potency of honey.

The preliminary results from this study demonstrate antibacterial potential of Sidr and Damas honey against *S. aureus*. Further studies involving more samples and test organisms could assess the potential application in complementary medicine and food preservation.

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