Antibacterial Properties of Local Texan Honey against Staphylococcus Aureus

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Abstract

This study is motivated by the notable absence of recorded resistant bacteria attributed to honey’s unique synergistic mechanism of action. Focusing on the escalating threat posed by multi-drug-resistant Staphylococcus aureus, a prominent member of the ESRAKE pathogens, the research aims to evaluate the effectiveness of locally sourced Texan honey in inhibiting bacterial growth. Employing Mueller-Hinton agar plates as the experimental platform, the investigation goes into the intricate relationship between honey’s compositional attributes—namely, moisture content, pH levels, and hydrogen peroxide activity—and the resulting zones of inhibition (ZOI). A total of 36 Texan honey samples were analyzed and the aforementioned parameters were quantified. 86% of the honey samples exhibited ZOI values exceeding the literature standard of 16 mm, indicating robust antibacterial properties. Additionally, 11 of them (31%) had a water content of 18% or lower, which is congruent with honey’s literary moisture content. Based on the results collected, an inverse relationship was found between honey’s water content and its measured ZOI. These findings underscore honey’s potential as a viable therapeutic agent for illnesses instigated by ESRAKE pathogens as well as address the urgent global challenge of antibiotic resistance. Further research is recommended to deepen the application of local Texan honey as an effective and cost-efficient alternative to treat bacterial infections.

Introduction

Honey, an ancient marvel produced by bees, is renowned for its medicinal potential, notably as an antibiotic. This study delves into the antibacterial properties of honey, focusing on 12 Texan samples and their efficacy against the ESRAKE pathogen Staphylococcus aureus. These pathogens, resistant to conventional antibiotics, pose a global health threat. Understanding their resistance mechanisms is crucial. Texas, with its diverse flora, provides an ideal backdrop for studying honey’s potential, notably as an antibiotic. This study delves into the antibacterial properties of honey, focusing on 12 Texan samples and their efficacy against the ESRAKE pathogen Staphylococcus aureus. These pathogens, resistant to conventional antibiotics, pose a global health threat. Understanding their resistance mechanisms is crucial. The University of Texas at San Antonio, San Antonio TX, 78249

Materials and Methods

For the Zone of Inhibition (ZOI) testing, Mannitol Salt Agar with 7.5% NaCl was utilized along with overnight-grown Staphylococcus aureus cultures, Mueller-Hinton 0.5 Standard, undiluted honey samples (100%), a large Petri dish, 70% ethanol for cleaning, micropipettes, 1000 µl micropipette tips, toothpicks, autoclaved beads for cell spreading, a Bunsen burner, and Parafilm. The procedure involved stringent cleanliness measures, plate labeling, bacterial density standardization, inoculation, honey application, photographing, and incubation at 37°C for 24 hours. After incubation, the ZOI of each honey sample was measured, and the average was calculated. For the H2O2 analysis, materials such as 50% (w/v) honey solution, HRP enzyme, dianisidine substrate, phosphate-buffered saline (PBS), sulfuric acid (H2SO4), microtube tubes, a microplate reader, and hydrogen peroxide (H2O2) solutions were employed. The process involved generating a standard curve, diluting honey samples, adding colorimetric reagents, stopping the reaction, washing, and measuring absorbance to determine HRP activity. Additionally, pH and moisture content were assessed using honey samples, DI water, a pH probe, a refractometer, centrifuge tubes, a vortex, and a scale. Dilution, pH measurement, and moisture determination were conducted according to specified procedures for each sample.

Results

For the Zone of Inhibition (ZOI) testing, Mannitol Salt Agar with 7.5% NaCl was utilized along with overnight-grown Staphylococcus aureus cultures, Mueller-Hinton 0.5 Standard, undiluted honey samples (100%), a large Petri dish, 70% ethanol for cleaning, micropipettes, 1000 µl micropipette tips, toothpicks, autoclaved beads for cell spreading, a Bunsen burner, and Parafilm. The procedure involved stringent cleanliness measures, plate labeling, bacterial density standardization, inoculation, honey application, photographing, and incubation at 37°C for 24 hours. After incubation, the ZOI of each honey sample was measured, and the average was calculated. For the H2O2 analysis, materials such as 50% (w/v) honey solution, HRP enzyme, dianisidine substrate, phosphate-buffered saline (PBS), sulfuric acid (H2SO4), microtube tubes, a microplate reader, and hydrogen peroxide (H2O2) solutions were employed. The process involved generating a standard curve, diluting honey samples, adding colorimetric reagents, stopping the reaction, washing, and measuring absorbance to determine HRP activity. Additionally, pH and moisture content were assessed using honey samples, DI water, a pH probe, a refractometer, centrifuge tubes, a vortex, and a scale. Dilution, pH measurement, and moisture determination were conducted according to specified procedures for each sample.

Conclusions & Future Research

TX Hones: •66% of Texan honey samples surpassed the 16 mm benchmark for antibacterial activity against Staphylococcus aureus. •Lower moisture content correlates with stronger inhibitory effects. •pH range of 3 to 4 enhances antimicrobial activity. •Hydrogen peroxide activity showed no direct correlation with inhibition. •Potential for honey as a cost-effective antimicrobial alternative.

Future Applications: •Potential application on other ESRAKE pathogens.

References


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Figure 1. Graph showing zone of inhibition of honey samples against Staphylococcus aureus.

Figure 2. ZOI of honey samples against Staphylococcus aureus vs. water content of honey.

Figure 3. ZOI of honey samples against Staphylococcus aureus vs. hydrogen peroxide activity of honey.

Figure 4. Zone of inhibition (ZOI) measurements for a few of the samples used in this study.