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## Abstract

Reactive oxygen species (ROS) are unstable molecules that drive oxidative stress, a major contributor to chronic diseases such as cardiovascular disease, neurodegenerative disorders, and cancer. Antioxidants play a critical role in neutralizing ROS, reducing cellular damage, and supporting overall health. Honey, a natural product valued for its antioxidant and antimicrobial properties, has drawn significant scientific interest. Manuka honey, in particular, has been widely studied and is recognized for its high antioxidant and antibacterial activity.

Spotted Lanternfly (SLF) honey, however, represents a new and largely unexplored resource. To date, no comprehensive evaluation of SLF honey's bioactive properties has been conducted. This study provides the first investigation into SLF honey as a U.S.-based honey with potentially comparable antioxidant and antimicrobial properties to Manuka honey. By assessing its biological activity, this research aims to contribute new insights into SLF honey's therapeutic potential, with implications for future applications in medicine and health.

## Introduction

Oxidative stress, caused by an excess of reactive oxygen species (ROS), plays a major role in the development of chronic conditions such as cancer, neurodegenerative disease, diabetes, and cardiovascular disorders.<sup>1-3</sup> While low levels of ROS are essential for immune function and cellular signaling, chronic imbalance leads to cellular damage.<sup>1,2,4</sup> To combat this, the body utilizes endogenous antioxidants and benefits from natural dietary sources.<sup>2,4,5</sup> Due to safety concerns surrounding synthetic antioxidants, attention has turned to natural compounds with therapeutic potential.<sup>4,5</sup>

Honey has emerged as a promising agent, with documented antioxidant, antibacterial, anti-inflammatory, and antifungal properties.<sup>6</sup> Manuka honey, produced in New Zealand and Australia, is the most studied medical-grade honey due to its high polyphenol content and potent bioactivity.7

Spotted Lanternfly (SLF) honey is a novel U.S.-based honey first identified in Pennsylvania in 2014. Beekeepers observed unusually dark honey containing tree-of-heaven sap and lanternfly honeydew.<sup>8</sup> Darker honeys are known to correlate with higher phenolic content and antioxidant capacity. <sup>9</sup> Despite its distinctive composition, SLF honey has not been studied extensively.

Our research provides the first formal evaluation of SLF honey's antioxidant and antibacterial properties. We hypothesize that SLF honey, due to its dark color and unique bioactive profile, may exhibit activity comparable to or greater than Manuka honey, positioning it as a promising U.S.-based candidate for medical-grade applications.



Figure 1. Picture showing Spotted Lanternfly insect.

# **Spotted Lanternfly (SLF) Honey as a Strong Candidate** for U.S. Based Medical-Grade Honey

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## Objective

- The long-term goal of this study is to identify medical grade honeys in the U.S. to be used in the medical field.
- The overall objective of this study is to assess the antibacterial and antioxidant potential of SLF honey through standardized antioxidant and antibacterial assays to determine its suitability for consideration as a U.S.based medical-grade honey.

## Materials and Methods

#### **SLF Honey Samples**

78 Honey samples, including 5 non-SLF controls, were collected in 2023-2024 from beekeepers in Pennsylvania. The samples were analyzed through QPCR at the Honey and Pollen Diagnostics Lab at Penn State University, where they were confirmed to be Spotted Lanternfly (SLF) honey. These samples were kindly donated by Dr. Robyn Underwood from the Penn State Extension Lab for use in our research study.

#### **DPPH Radical Scavenging Assay**

A 0.06 mM 2,2- diphenyl-1-picrylhydrazyl (DPPH) solution was prepared and refrigerated. Seventy-eight honey samples (three controls and 75 SLF) were dissolved in deionized water to a 10% w/v honey solution. In a 96-well plate, 20 µL of each sample was mixed with 180 µL of DPPH solution. Methanol and DPPH were used as the blank and control, respectively. After 30 minutes of incubation at 25°C in the dark, absorbance was measured at 517 nm. Radical scavenging activity (RSA) was calculated as the percentage of scavenged DPPH radicals (%RSA).

#### Folin-Ciocalteu Assay (TPC)

Honey samples (10% w/v) and gallic acid standards were prepared. Aliquots of 100 µL of each sample were mixed with Folin–Ciocalteu reagent and sodium carbonate, then incubated at 25°C for 2 hours in the dark. The mixtures were transferred to a 96-well plate, and absorbance was measured at 765 nm. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (GAE) per kilogram of honey.

#### Agar Well Diffusion Assay

Staphylococcus aureus cultures were diluted in Mueller-Hinton Broth (MHB) to a 0.5 McFarland standard (~10<sup>8</sup> CFU/mL). A 1:5 dilution was prepared and visually confirmed. Then, 100 µL of the suspension was spread evenly onto Mueller-Hinton agar (MHA) plates. Wells were created using a sterile pipette tip, and undiluted honey samples were applied using sterile toothpicks; control wells were left empty. Plates were incubated overnight at 37°C, and zones of inhibition (ZOI) were measured in millimeters.

## Results

#### Antioxidant Activity - DPPH Radical Scavenging Assay

The antioxidant potentials of the SLF and Manuka honey samples were compared using the DPPH free radical scavenging assay. Absorbances were measured at 517 nm, and the percent radical scavenging activity (%RSA) was calculated relative to control (DPPH solution). The results indicated high radical scavenging activity among the Spotted Lanternfly (SLF) honey samples (Figure 1). The highest radical scavenging activity was observed in sample PA3, with a %RSA of 92.804%. The lowest activity in SLF honey samples was exhibited by sample Wiles 224, with a %RSA of 11.663%.

#### Antioxidant Potential (Total Phenolic Contents - TPC)

The TPC results for Spotted Lanternfly honey demonstrated variability among samples; however, the majority exhibited high total phenolic content (Figure 2). The highest TPC was observed in sample NJ-14, with a gallic acid equivalent (GAE) of 211.101 mg/100 g honey. Conversely, sample CAT-1 exhibited the lowest TPC among SLF honeys, with a GAE of 4.585 mg/100 g honey.

## **Results – con't**

#### **Antibacterial Activity – Zone of Inhibition**

The antibacterial potential of the SLF honey samples against S. aureus was assessed using the agar well diffusion method by measuring the ZOI. Most SLF honey samples exhibited strong antibacterial activity against S. aureus (Figure 4 and 5). The largest zone of inhibition was observed for sample NJ-15 (26.02 mm), whereas the smallest zones were recorded for samples AB-32, AR-37, AR-42, C1, and C2 (9.00 mm).



🗆 ZOI 9-11.99 mm ZOI 12-15.99 mm ZOI 16-19.99 mm ZOI 20-23.99 mm ZOI 24-27.99 mm

Figure 4. Pie chart showing the distribution of SLF honey samples' zones of inhibition against S.aureus, grouped into defined ranges; each slice indicates the count of samples in that range.



Figure 5. Pie chart showing the distribution of Manuka honey samples' ZOI against S.aureus, grouped into defined ranges; each slice indicates the number of samples in that range.

## Conclusions

- 56.6% of SLF samples (43 samples out of 76) have a ZOI greater than 16 mm suggesting that the majority of SLF honeys exhibit strong antibacterial activity against S. aureus that is comparable to or greater than Manuka honey, indicating their potential use in future medical applications for the prevention and treatment of S. aureus-associated infections.
- 97.4 % of SLF samples (76 out of 78) have a %RSA greater than 20 and 66.7% of SLF samples (52 out of 78) have a TPC above 91 GAE. This suggests high antioxidant potential of SLF honeys indicate that they hold promise for prevention and management of oxidative-stress related diseases.
- Overall high bioactivity levels of SLF honey makes it a strong candidate to be identified as U.S. based medical grade honey.

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