

Abstract

We examined more than 100 local Texas honey samples for their antimicrobial and antioxidant properties. We identified some honeys with greater antioxidant potential, presented higher antimicrobial capacity comparable with current medical-grade honeys. Although several studies have analyzed honey's antimicrobial activity against numerous gram-positive and gram-negative bacteria, the effect on Cutibacterium acnes remains unknown. This study analyzed the antimicrobial capacity of local Texas honeys against C. acnes in terms of bactericidal and bacteriostatic activity.

Introduction (Background)

Honey's extensive benefits for skin care have been recognized for centuries, owing to its high biological activity. It regulates bacteria growth and aids skin homeostasis, while also functioning as an antibacterial, anti-inflammatory, anti-aging, and antioxidant agent [1], thus reducing the development of clogged pores. Acne, an inflammatory skin disorder, occurs when sebaceous glands become clogged due to bacteria invading the skin's surface, leading to increased Cutibacterium acnes (C. acnes) colonies and resulting in pimples and blackheads on the face, back, and chest [2]. Despite various available acne treatment modalities, such as topical and oral antibiotic therapy, which are recommended to combat acne pathogenesis, they can lead to adverse side effects, including bacterial resistance. The emergence of multidrug-resistant bacteria (MDR) has become a global problem, prompting significant efforts to find alternative therapies. Studies indicate that honey has a low emergence of bacterial resistance due to variations in composition arising from nectar sources, climate conditions, storage duration, diverse floral origins, and preservation conditions [3]. While honey is renowned for its antimicrobial properties and wound-healing effects, its application to inflammatory acne remains insufficiently studied. Thus, exploring the dermatological application of honey could pave the way for developing effective, low-cost treatments for acne vulgaris without adverse side effects.



Honey vs. Cutibacterium Acnes

Methods

We collected 117 honey samples between 2021 and 2022 from beekeepers of various geographical locations throughout Texas. The biological activity levels (BALs) of the honey samples were determined through antioxidant, antimicrobial, and physicochemical analyses using the biochemical and microbiological techniques at the UTSA HONEY Analysis Lab. The honey samples with the highest BAL values (n:15) were selected for this study of C. acnes inhibition.

The antimicrobial activity of these high BAL honey was analyzed using the agar well diffusion method for their ability to inhibit C. acnes proliferation. Zone of inhibition was measured after 7 days of anaerobic incubation at 37°C.



Figure 3. Agar well-diffusion Assay

Results

The agar well-diffusion test results showed that certain honey varieties have strong antimicrobial properties against C. acnes, making them promising for treating acne. However, the size of the clear zones of inhibition varies among different honey samples tested. The honey samples ranged in color from white to dark which was determined using a spectrophotometer amber, measuring the absorbance at 560nm and multiplying by a factor of 3.15. The H2O2-based antimicrobial activity of honey can be affected by storage and processing conditions that affect glucose oxidase (an enzyme introduced into nectar by worker bees), which is vulnerable to damage from heat and light making it highly variable.

tweezers.



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Figure 4. Plate 1 SA samples: 20, 32, 30, 21, 23. Plate 2 SA samples: 6, 33, 16, 37, 4. Plate 3 SA samples: 19, 36, 17, 27, 5.

After a total of 7 days (168 hours) of anaerobic incubation period at 37° C, all 15 honey samples showed ZOI ranges. The largest ZOI in order is SA-5 (27.15 mm), SA-4 (25.89 mm), SA-37(21.55 mm), SA-16 (19.29 mm), and SA-33 (18.78 mm).

Zone of Inhibition vs Hydrogen Peroxide Activity



Figure 5. The honey samples with a ZOI greater than 16 mm indicated high antimicrobial activity against C. acnes. Among the honey samples tested, SA-5 had the largest zone of inhibition, indicating superior antimicrobial activity. SA-5 has no peroxide activity despite recording the largest ZOI (27mm). H_2O_2 varies due to the type of honey, age, storage conditions, and honey dilutions.



Figure 6. SA-5, SA-37, and SA-4, were classified as Dark Amber and these samples also recorded ZOI greater than 20mm. This suggests that darker honey varieties contain greater mineral content and active pigments, such as carotenoids and flavonoids, which contribute to their dark color and superior antimicrobial properties.

Conclusions

- C.acnes is an anaerobic gram-positive pathogen that took 7 days to grow in an anaerobic chamber; thus, considering every variable was crucial to the outcome
- C. acnes growth curve were optimized with different concentrations of starting bacteria using the OD600 measurements.
- The top 5 honey samples with the largest ZOI were SA-5, 19, 37, 16, and 33, which is originated from South-Central and Texas Hill country regions
- Dark amber and Amber honeys have superior antimicrobial properties
- Lighter color honey like SA-36 and SA-27 had a low pFund value with high hydrogen peroxide activity.
- Honey samples that had a large ZOI (>16 mm) will be tested for Minimum Inhibitory Concentration (MIC) and Maximum Bactericidal Concentration (MBC).

Broader Impacts

- Natural non-invasive approach can create a platform to establish novel dermatological treatment plans for acne using local medical-grade honeys
- Formulation of medical-grade honey-based products and their application in human clinical trials will help with dryness, redness, scarring, and skin irritation as it changes the management of acne treatment.
- Honey-based acne treatment provides a cost-effective alternative to conventional treatments such as topical retinoids and hormonal regimens
- Advanced honey-derived skin products may decrease skin sensitivity for acne treatment.
- Chemical and biological components of the medicalgrade honey can be identified using biochemical tools such as GCMS, LCMS, HPLC etc.

References

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Acknowledgements

The funding for this research was provided by the UTSA Department of Integrative Biology SBRI Program and HONEY Pathway funded by NextGen program of USDA-NIFA. The honey samples were generously donated by Texas beekeepers. Special thanks to Steven Quezada for his lab assistance during bacterial growth and Dr. Ferhat Ozturk for guiding and mentoring us throughout the experimental process.