Antibacterial Efficacy and Saccharide Composition of Honey Determined by Microbial Assays and GC-MS

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Abstract

- Honey has been used for centuries as a natural remedy and is being explored as an alternative antibacterial agent due to rising antibiotic resistance.[1]
- Initial antibacterial testing was performed using 10-fold serial dilutions for honeys and antibiotics.[2]
- A two-step derivatization process enabled GC-MS analysis to identify saccharides using retention index values.[3]
- Heavy metal content was also analyzed to further compare honey types and their antibacterial properties.[4]
- Results contribute to understanding honey's potential as an alternative therapeutic agent and highlight the need for further research.

Background

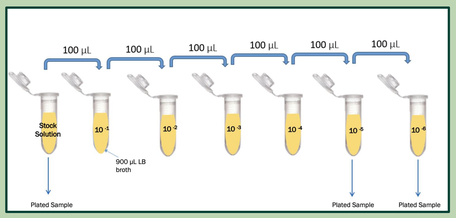
- Antibiotics have long been the first line of treatment for bacterial infections; however, the rise of antibiotic resistance has become a major public health concern.
- Honey has been used for centuries as a natural remedy and has recently gained attention for its potential antibacterial properties.
- Honeys antibacterial activity is attributed to its high sugar content, low pH, and the presence of hydrogen peroxide, which ultimately contribute to bacterial cell death.[5]
- Honeys inhibitory effects work against both gram-positive and gram-negative bacteria, indicating it can be used against a variety of pathogens.[6]
- These factors that honey as an antibiotic can work in multiple pathways, proposing the idea that honey has the potential to mimic the performance of traditional antibiotics.

Acknowledgements

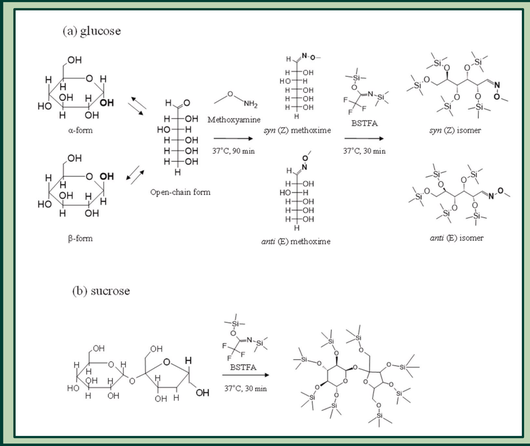
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Methods

Microbial Assay Using Serial Dilutions



- Multiple serial dilutions were completed for raw and commercial honeys, along with the antibiotic samples (ampicillin and kanamycin).
- Colony forming units (CFUs) of *E. coli* and *B. subtilis* were observed.



GC-MS Following Derivatization

- A two-step derivatization process was performed to prepare sugars for GC-MS.
  - Sugars undergo oximation, then are treated with BSTFA.
- Fructose, glucose, and sucrose are identified using retention index values as compared to standards.

Heavy Metal Testing Using the EPA 3050B Method



- Samples were digested using nitric acid and hydrogen peroxide.
- Heavy metal concentrations were analyzed using ICP-OES.
- Lead, cadmium, zinc, arsenic, nickel, chromium, and copper were targeted using standard solutions.

Results

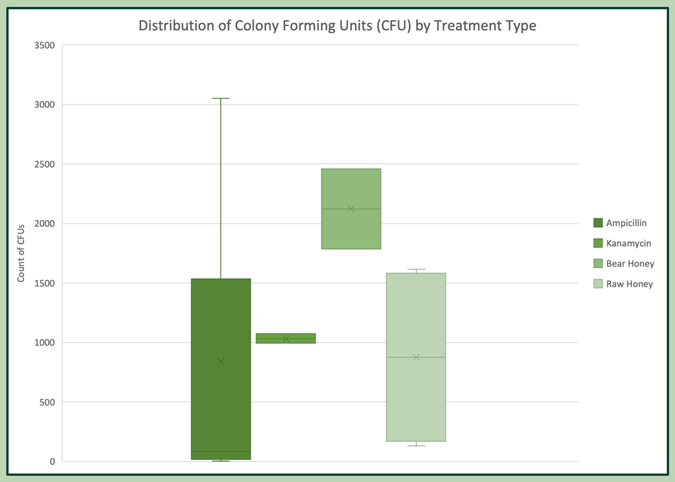


Figure 1: Distribution of colony forming unit counts by treatment type following serial dilution assays.

	Count of CFUs
B. subtilis	**13**
Ampicillin	11
Bear Honey	2
E. coli	**12**
Ampicillin	6
Kanamycin	2
Raw Honey	4
Grand Total	**25**

Table 1: Distribution of plates with countable CFUs.



Figure 2: Sample bacterial plate with CFUs.

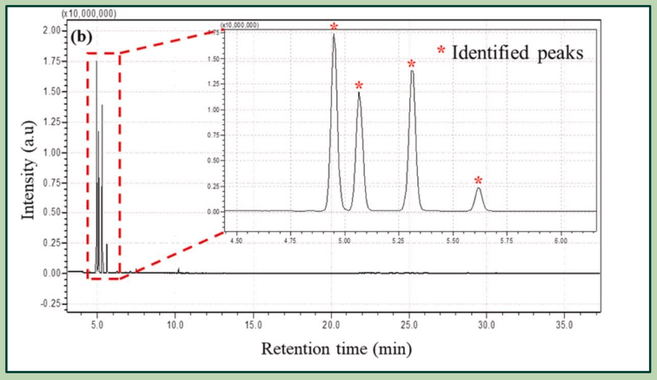


Figure 3: Expected GC-MS graph for raw honey sample. Note the high intensity peaks which represent fructose and glucose respectfully.[3]

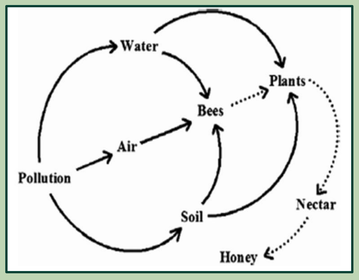


Figure 4: Schematic representing how heavy metals are incorporated into honey.[4]

Discussion

- Ampicillin produced the lowest CFU counts but showed the greatest variability, while kanamycin and commercial honey displayed minimal variation, with commercial honey having the highest CFU counts.
- Raw honey demonstrated consistent inhibition similar to ampicillin, suggesting potential antimicrobial activity against *E. coli*.
- Commercially available honeys may be adulterated with sugar substitutes, affecting the concentration of fructose and glucose. This decreases its antibacterial efficacy.
- Presence of heavy metals in honey varies by geographic origin, exposure to pollutants, and processing methods.

Conclusion

- Honey may have the potential to serve alongside traditional therapy and be an accessible, cost-effective treatment in modern medicine.
- Variability in sugar composition and heavy metal content serve as important standards for quality control in therapeutic use.
- Additional studies exploring synergistic effects between honey and antibiotics may also provide valuable insight.

References