# Evaluation of Antibacterial Activity of Two Different Honeys against Clinical Isolates of β-hemolytic Streptococci Group A

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## Abstract

**Introduction**: The aim of this investigation was to evaluate *in vitro* antibacterial activity of horse chestnut honey and acacia honey at different concentrations against clinical isolates of the β-hemolytic streptococci group A (BHS-A). Honey's active compounds have multiple therapeutic effects and it is used as traditional medicine for treatment and prevention of various illnesses.

**Materials and methods**: The antimicrobial effects of two honeys were tested on forty-four strains of BHS-A, isolated from the throat by a modified disk diffusion method. A bacterial suspension of BHS-A strains was plated onto *Müller-Hinton agar with 5% defibrinated horse blood.* Using a sterile 8 mm diameter cork borer, wells were cut in the agar and into each was introduced 100µL of the different concentrations of the honey solution (25 %v/v, 50 %v/v, 75% v/v and 100%v/v). A disk of penicillin was added as the positive control. The plates were incubated aerobically for18-24 hours at 36 (± 1)˚C and zones of inhibition were measured.

**Results**: The average diameter of the inhibition zones of acacia honey (100 %v/v) was 12.48 mm ± 1.73 mm, for acacia honey (75 %v/v) it was 11.06 mm±1.24 mm and for horse chestnut honey (100 %v/v) it was 11.08 mm ± 1.02 mm. The positive control showed the average diameter of 30.45 mm ± 3.21 mm. Statistical significance ($p < 0.05$) was observed comparing the zone diameters of the acacia honey (100%) and antibiotic penicillin, and between the horse chestnut honey (100%) and antibiotic penicillin.

**Conclusion**: Acacia and horse chestnut honeys exhibit limited but effective antibacterial activity upon clinical isolates of BHS-A.

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## Introduction

β-hemolytic streptococci group A (BHS-A) are the most common bacterial cause of tonsillopharyngitis. This microorganism can also cause acute otitis media, pneumonia, skin and soft-tissue infections; cardiovascular, musculoskeletal, and lymphatic infections, bacteremia and meningitis (1). Classic streptococcal tonsillopharyngitis has an acute onset; produces concurrent dysphagia, headache, and upon examination is characterized by intense tonsillopharyngeal erythema, white-yellow exudates, and tender/enlarged palatine tonsils. Patients with acute streptococcal tonsillopharyngitis should receive therapy with an antimicrobial agent in a dose and for a duration that is likely to eradicate the infecting organism from the pharynx. Most oral antibiotic therapy must be administered for the conventional 10 days to achieve maximal rates of pharyngeal eradication of BHS-A (2-3).

Over the past decade, several research groups have focused their attention to the use of bee honey as a supplement therapy (4-6). The use of bee honey as a traditional remedy for microbial infections dates back to ancient times (7). Aristotle, c.350 BC recommended that bee honey collected in specific regions and seasons (and therefore presumably from different floral sources) can be used for the treatment of different ailments (8). Certain types of honey exhibit broad-spectrum antimicrobial activity and are effective against antibiotic resistant bacterial pathogens (9-13). The present study aimed to evaluate the antibacterial activity of two bee honey varieties (horse chestnut honey and acacia honey) onto forty-four clinical isolates of BHS-A.

## Materials and Methods

*Bacterial strain*

Forty-four clinical isolates of beta-hemolytic streptococci group A (BHS-A) were evaluated. All these clinical isolates were donated by the Microbiology Department of the Public Health Institute of the County Brodsko-Posavska. Isolates of BHS-A used in our study were collected from throat swabs and were positive to BHS-A. They were identified on the basis of zone diameter around a bacitracin disk (10 µg/disk) as well as on susceptibility testing and by a latex agglutination slide test for the grouping of Streptococci of the Lancefield groups A, B, C, D, F and G from culture plates as recommended by CLSI (14).

*Honey samples and dilution of honey*

Two honey varieties were used: horse chestnut honey and acacia honey, which were purchased from the local market from local beekeepers. Because of the intensified viscosity of acacia honey, different concentrations were made in sterile distilled water: 25 % v/v, 50 % v/v, 75 % v/v. This was done by dissolving the respective volumes: 8.3 ml, 16.6 ml and 25 ml of acacia honey, into corresponding volumes of sterile distilled water to give a 33.33 ml preparation. These were the tested different concentrations in addition to non-diluted horse chestnut honey, acacia honey and penicillin disk as the control.

*Susceptibility testing of honey*

A bacterial suspension of BHS-A strains, equal to the McFarland standard of 0.5 was prepared in saline. With sterile swabs (Copan Italia S.p.A, Brescia, Italy -plain swab sterile), the suspension was coated onto a Müller-Hinton agar with 5% defibrinated horse blood. Using a sterile 8 mm diameter cork borer, five wells were cut in the agar and into each was introduced 100µL of the different concentrations of the honey solution. Into the place of the sixth well, a disk of penicillin was added as the positive control. The plates were incubated aerobically for18-24 hours at 36 (± 1)°C and examined for zones of inhibition. The zones of inhibition were measured (in millimeters) and recorded.

***Table 1.*** *Inhibition zones of acacia honey and horse chestnut honey against beta-hemolytic streptococci group A (BHS-A) isolates.*

| Beta-hemolytic streptococci group A isolates | Acacia honey (concentration of honey) | | | | Horse chestnut honey 100% | Antibiotic-penicillin |
|---|---|---|---|---|---|---|
| | 100% | 75% v/v | 50% v/v | 25% v/v | | |
| BHS-A1 | 12 | 11 | 0 | 0 | 10 | 28 |
| BHS-A2 | 14 | 12 | 0 | 0 | 12 | 28 |
| BHS-A3 | 11 | 10 | 0 | 0 | 10 | 30 |
| BHS-A4 | 12 | 11 | 0 | 0 | 11 | 23 |
| BHS-A5 | 16 | 14 | 11 | 0 | 12 | 25 |
| BHS-A6 | 15 | 12 | 0 | 0 | 10 | 33 |
| BHS-A7 | 13 | 10 | 0 | 0 | 11 | 33 |
| BHS-A8 | 14 | 12 | 0 | 0 | 11 | 30 |
| BHS-A9 | 15 | 12 | 9 | 0 | 12 | 33 |
| BHS-A10 | 12 | 11 | 0 | 0 | 11 | 28 |
| BHS-A11 | 13 | 9 | 0 | 0 | 0 | 28 |
| BHS-A12 | 12 | 11 | 0 | 0 | 0 | 34 |
| BHS-A13 | 12 | 12 | 0 | 0 | 11 | 31 |
| BHS-A14 | 18 | 13 | 10 | 0 | 13 | 33 |
| BHS-A15 | 12 | 11 | 0 | 0 | 11 | 32 |
| BHS-A16 | 15 | 11 | 0 | 0 | 0 | 27 |
| BHS-A17 | 15 | 14 | 0 | 0 | 12 | 31 |
| BHS-A18 | 11 | 11 | 0 | 0 | 11 | 32 |
| BHS-A19 | 0 | 0 | 0 | 0 | 0 | 25 |
| BHS-A20 | 10 | 0 | 0 | 0 | 0 | 28 |
| BHS-A21 | 12 | 10 | 0 | 0 | 0 | 26 |
| BHS-A22 | 12 | 11 | 0 | 0 | 10 | 32 |
| BHS-A23 | 12 | 12 | 0 | 0 | 10 | 29 |
| BHS-A24 | 0 | 9 | 0 | 0 | 0 | 27 |
| BHS-A25 | 11 | 0 | 0 | 0 | 11 | 31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| BHS-A26 | 11 | 11 | 0 | 0 | 0 | 33 |
| BHS-A27 | 11 | 0 | 0 | 0 | 0 | 28 |
| BHS-A28 | 15 | 13 | 0 | 0 | 13 | 31 |
| BHS-A29 | 11 | 0 | 0 | 0 | 10 | 32 |
| BHS-A30 | 12 | 0 | 0 | 0 | 10 | 30 |
| BHS-A31 | 11 | 10 | 0 | 0 | 0 | 32 |
| BHS-A32 | 12 | 9 | 0 | 0 | 0 | 33 |
| BHS-A33 | 13 | 10 | 0 | 0 | 0 | 26 |
| BHS-A34 | 12 | 0 | 0 | 0 | 0 | 34 |
| BHS-A35 | 12 | 10 | 0 | 0 | 0 | 32 |
| BHS-A36 | 12 | 11 | 0 | 0 | 0 | 29 |
| BHS-A37 | 13 | 11 | 0 | 0 | 0 | 40 |
| BHS-A38 | 10 | 10 | 0 | 0 | 0 | 32 |
| BHS-A39 | 10 | 0 | 0 | 0 | 0 | 33 |
| BHS-A40 | 12 | 10 | 0 | 0 | 13 | 31 |
| BHS-A41 | 11 | 10 | 0 | 0 | 10 | 28 |
| BHS-A42 | 13 | 12 | 0 | 0 | 11 | 36 |
| BHS-A43 | 11 | 11 | 0 | 0 | 0 | 30 |
| BHS-A44 | 13 | 11 | 0 | 0 | 0 | 33 |

**The zones of inhibition were measured in millimeters*

*Statistical analysis*

Statistical analysis was performed using the program SigmaPlot Version 12.0. The differences observed between the groups were tested by multiple comparison procedures (Turkey test). The statistical significance level was confirmed at $p < 0.05$.

## Results

The activity of acacia honey and horse chestnut honey against BHS-A isolates and the inhibitions zones are shown in Table 1. The average diameter of the inhibition zones of acacia honey (100 % v/v) on strains that were sensitive to the honey was 12.48 mm (standard deviation - SD = 1.73 mm). The average diameter of the inhibition zones of acacia honey (75 % v/v) on strains that were sensitive to the honey was 11.06 mm (SD = 1.24mm). Finally, the average diameter of the inhibition zones of horse chestnut honey (100 % v/v) on strains that were sensitive to the honey was 11.08 mm (SD = 1.02 mm). Antibiotic penicillin showed the average diameter of the inhibition zones to be 30.45 mm (SD = 3.21mm). Comparing the zone of inhibitions, by multiple comparison procedures, of different concentrations of

honeys and penicillin (positive control), astatistically significant difference was observed with 100% acacia honey and100 % horse chestnut honey ($p < 0.05$).

## Discussion

Honey has several properties that contribute to its antimicrobial activity. Low pH and high osmolarity are combined with the enzymatic production of hydrogen peroxide that exerts an antimicrobial effect (15-16). Hydrogen peroxide is the major contributor to the antimicrobial activity of honey (17). The different concentrations of this compound in different honeys result in their varying antimicrobial effects (7).

In our research, we evaluated the antimicrobial activity of honey on BHS-A because, if in the case of positive antibacterial activity of honey on the BHS-A, it can be used primarily in the supportive therapy for e.g. tonsillopharyngitis. The use of honey is extremely uncomplicated and easily applicable.

Researchers who evaluated the antimicrobial effect of honey showed that certain microorganisms have better sensitivity to it. Mittal et al. reported greater honey activity in vitro against *Staphylococcus aureus,* and *Escherichia coli* than on *Pseudomonas aeruginosa* (18). Cooper et al. reported honey activity on catalase negative, gram positive cocci (5); while Hannan et al. reported honey activity on typhoidal Salmonellae.

When observing the antimicrobial activity of honey, the key element of effectiveness is the type of honey. Certain types of honey, such as Manuka honey, exhibit extensive antimicrobial activity. A group of researchers from Ireland and Australia reported that Manuka honey has clinical potential and a greater antimicrobial effect in vitro against *Staphylococcus aureus,* and *Escherichia coli* than on *Pseudomonas aeruginosa* (19, 20). A group of researchers from Cameroon focused on the antimicrobial activity of honeys on clinical isolates of *Helicobacter pylori* (6, 21, 22). The results of such research can provide alternative therapies against certain bacteria.

In our research, it was observed that 100% of acacia honey and horse chestnut honey show maximum antimicrobial activity as shown in Table 1. When the concentrations of honey were 50% v/v and 25% v/v there was not an antimicrobial effect. This conclusion of our study is inconsistent with the conclusion of other authors (6). It is assumed that such differences are due to the various kinds of honeys.

In this study, in order to evaluate the antibacterial activity of two honey varieties (horse chestnut honey and acacia honey) upon clinical isolates of BHS-A, we concluded that honeys exhibit limited, but effective antibacterial activity. Such results support the use of honey as a supplement therapy for BHS-A infections e.g. tonsillopharyngitis. Further research is required to determine the *in vivo* activity of horse chestnut honey and acacia honey onto BHS-A.

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## Disclosure

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**Competing interests.** None to declare.

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