Synergistic Action of Starch and Honey Against *Pseudomonas aeruginosa* in Correlation with Diastase Number

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**ABSTRACT**

To evaluate the synergistic action of starch on the antibacterial activity of honey against *Pseudomonas aeruginosa*, a comparative method of adding honey with and without starch to culture media was used. *P. aeruginosa* (ATCC 27853) was used to determine the minimum inhibitory concentration (MIC) of five varieties of honey. In a second step, lower concentrations of honey than the MIC were incubated with a set of concentrations of starch and then added to media to determine the minimum synergistic inhibitory concentration. The MIC for the five varieties of honey without starch against *P. aeruginosa* ranged between 15% and 26% (vol/vol). When starch was incubated with honey and then added to media, a significant MIC drop has been noticed with each variety and it ranged between 30.7% and 46.6%. No significant correlation has been established between the MIC drop and the diastase number.

**INTRODUCTION**

Honey has a potent antibacterial activity and is very effective in clearing infection in wounds and protecting them from becoming infected.\(^1\) It has been demonstrated in many studies that honey has antibacterial effects, attributed to its high osmolarity, low pH, hydrogen peroxide content and content of other, uncharacterised compounds.\(^2\) The low water activity of honey is inhibitory to the growth of the majority of bacteria, but this is not the only explanation for its antimicrobial activity. Molan\(^3\) has studied sugar syrups of the same water activity as honey and found them to be less effective than honey at inhibiting microbial growth *in vitro*. Unlike honey, no inhibitory action has been allocated to starch. However, it is known that starch could be incorporated into microbial media to stimulate their growth.\(^4\) One of the characteristics that set honey apart from all other sweetening agents is the presence of enzymes. Some of the most important honey enzymes are invertase, diastase, and glucose oxidase. Those most prominent are added by the bee during the conversion of nectar to honey. In some countries, the specification of enzymes is a binding legal indicator.\(^5\) The diastase content varies according to floral source, long storage periods, and exposure to high temperatures.\(^6\) Legislation has set a minimum level for diastase activity; it should not be less than 8 Diastase Number (DN) units, where 1 DN unit hydrolyses 1 mL of 1% starch using 1 g of honey for 1 hour at 37°C.\(^7\) The α-amylase splits the starch chain randomly, producing dextrin, and the β-amylase splits the reducing sugar maltose from the ends of the starch chain,\(^8\) but no starch is found in honey. Therefore, it is expected that adding starch, which is the substrate of the diastase, to honey will subsequently increase the antibacterial effect of honey. This study was carried out to evaluate the antibacterial properties of honey and starch when used jointly to manage superficial wounds. On the other hand, we are exploring a novel concept that starch, normally a microbial nutrient, may actually enhance the antibacterial properties of honey. A possible correlation between the additive action of honey and starch and the DN of honey was examined.

**MATERIALS AND METHODS**

*Honey samples*

Five varieties of honey (V1–V5) from different botanic origins (V1: multifloral; V2: eucalyptus; V3: orange; V4: jujube,
and V5: thistle) were analysed. Samples were obtained directly from beekeepers in different regions of Algeria during the year 2005. The Diastase Number (DN) of each variety was measured according to the Phadebas method.9,10

**Bacterial strain and inoculum standardization**

*Pseudomonas aeruginosa* (ATCC 27853) was kindly provided by the “Institut Pasteur d’Alger.” It was maintained by subculture in King A broth media. Prior to experiment the strain was inoculated into nutrient agar media. The inoculum suspensions of *P. aeruginosa* were obtained by taking five colonies from 24-hour-old cultures. The colonies were suspended in 5 mL of sterile saline (0.85% NaCl). The inoculum suspensions were shaken for 15 seconds and the density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1–5 × 10^6 cfu/mL) with sterile saline. The suspensions were diluted 1:1000 in RPMI 1640 to give a final inoculum suspension equivalent to 0.5–2.5 × 10^3 cfu/mL.

**Minimum inhibitory concentration measurement (MIC)**

Concentrations of honey between 10% and 30% (vol/vol) were incorporated into nutrient agar media to test their efficiency against *P. aeruginosa*. The final volume of honey and media in each plate was 5 mL. The plates were inoculated and incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was determined by finding the plates with the lowest concentration of honey on which the strain would not grow. All MIC values are expressed in percent (vol/vol).

In a second step and to evaluate the effect of starch on the antibacterial action of honey, a 10% starch solution was prepared using sterile water. Different volumes from the stock solution were added to a range of honey concentrations below the MIC. The same volume of starch solution that has given inhibition with honey is added alone to media as control. An equivalent volume of water was added to honey instead of starch solution to confirm that inhibition is not due to the dilution of honey. The final volume in each plate was 5 mL. Starch content in media that gave syner-

<table>
<thead>
<tr>
<th>Honey varieties</th>
<th>MIC % (vol/vol) honey only</th>
<th>Honey content in media % (vol/vol)</th>
<th>Starch content in media % (vol/vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>15</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>V2</td>
<td>18</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>V3</td>
<td>26</td>
<td>18</td>
<td>1.9</td>
</tr>
<tr>
<td>V4</td>
<td>25</td>
<td>17</td>
<td>2.8</td>
</tr>
<tr>
<td>V5</td>
<td>22</td>
<td>13</td>
<td>2</td>
</tr>
</tbody>
</table>

**FIG. 1.** Isobologram representing overadditive effect of starch and variety 1 of honey against *Pseudomonas aeruginosa*.

**FIG. 2.** Isobologram representing overadditive effect of starch and variety 2 of honey against *Pseudomonas aeruginosa*. 
gistic action with honey varied within varieties and it ranged between 0.3% and 3% (wt/vol). Honey and starch as well as honey and water were incubated for 24 hours at 37°C before being incorporated into media. This will allow the diastase present in honey to act against starch. Plates were inoculated and incubated at 37°C for 24 hours. All inoculations were carried out in triplicates.

**Statistical analysis**

Isobolographic analysis was carried out to measure the additive action of honey and starch against the tested bacteria. Statistica® software (StatSoft, Tulsa, OK) was used to determine whether there is a correlation between the additive action and the DN.

**RESULTS**

Table 1 represents the DN of the five varieties of honey. The DN ranged between 13.1 and 26.1, which are in the interval of international standards. All varieties of honey were effective against *P. aeruginosa*. Without starch, the MIC of the five varieties ranged between 15% (vol/vol) and 26% (vol/vol) (Table 2). When starch was incubated with honey and added to media, the MIC of the five varieties ranged between 8% (vol/vol) and 18% (vol/vol), which represents a MIC drop between 30.7% and 46.6%. The inhibitory action was seen neither in the media containing starch only nor in media with honey and water. Statistically, additive effect is noticed between starch and all varieties of honey.
This additive effect has no significant correlation with DN ($R = 0.442$).

**DISCUSSION**

*P. aeruginosa* is the predominant cause of fatal burn wound sepsis,11 and isolation of multidrug-resistant strains is a common problem in hospitals. With increasing interest in the use of alternative therapies and as the development of antibiotic-resistant bacteria spreads, honey may finally receive its due recognition as a wound healer. Increasing interest has been recently accorded to the use of honey for managing wounds.12–15. Adding starch to honey has shown a significant decrease in the MIC for the five varieties of honey against the tested strain. Figures 1–5 show an additive action of starch and honey against the tested strain, which is represented by isobolograms. The figures show an overadditive effect of starch and all varieties of honey against *P. aeruginosa*. The isobolograms illustrate several different dose combinations that attain the specified effect level. The overadditive action is also called synergism.16 The inhibitory action was seen neither in the media containing starch only nor in media with honey and water with the corresponding MIC. Amylases present in honey were expected to split starch chains to randomly produce dextrin and maltose and probably increase the osmotic effect in the media by increasing the amount of sugars and consequently increase the antifungal activity. However, the amount of the amylase in honey is not in correlation with the relative potency of starch and honey (Fig. 6). That means that other unknown factors may play a role in this combined potency. Further research is needed to elucidate and optimize the effective combination of these natural products in clinical practice. The current prevalence of antibiotic-resistant microbial species has led to a reevaluation of the therapeutic use of ancient remedies, including honey and starch. Both natural products have no adverse effects on tissues, so they can be safely used on wounds and inserted into cavities and sinuses to clear infection.

**REFERENCES**


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