Original Research

Additive Activity of Royal Jelly and Honey Against *Pseudomonas aeruginosa*

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Abstract

As natural products garner attention in the medical field, the emergence of antibiotic-resistant strains of bacteria has confounded the current use of antibiotic therapy, leading to the re-examination of earlier remedies such as honey and royal jelly (RJ). Four varieties of honey and one variety of freshly reaped RJ were used to evaluate the additive antimicrobial action against *Pseudomonas aeruginosa* (ATCC 27853). Initially, honey and RJ were used separately to determine their minimum inhibitory concentration (MIC) against the tested strain. Next, sub-MIC concentrations of honey and RJ were incorporated into media to determine the minimum additive inhibitory concentration. When tested separately, the MIC of the four varieties of honey ranged from 12-18 percent (volume/volume; v/v), and that of RJ was 4 percent (v/v). When combined with RJ, each honey variety tested showed a greater than 90-percent drop in MIC using 3-percent (v/v) RJ, a 66.6-percent drop in MIC using 2-percent (v/v) RJ, and a 50-percent MIC drop with 1-percent (v/v) RJ. The MIC of RJ dropped by 75 percent when used with the half concentration of honey that alone provides the MIC and by 50 percent when used with one-third the concentration of honey that alone provides the MIC. A strong linear correlation exists between the MIC drop of each variety of honey and RJ. With increasing interest in the use of alternative therapies and as the development of antibiotic-resistant bacteria spreads, honey and RJ may receive renewed recognition as wound healers. (*Altern Med Rev* 2008;13(4):330-333)

Introduction

Pseudomonas aeruginosa is the predominant cause of fatal burn wound sepsis[1] and isolation of multi-drug resistant strains is a common problem in hospitals, leaving minimal or no effective systemic treatment options for the clinician. Increasing resistance of *P. aeruginosa* to many antibiotics has been observed and poses a therapeutic dilemma.[2]

Natural medicinal products have been used for millennia to treat multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently resurgence of interest by physicians in natural products. Honey and royal jelly (RJ) are complex heterogeneous mixtures of flower nectar sugars, proteins, and glandular secretions from bees. Honey has potent antibacterial activity and is effective in preventing and clearing wound infections.[3] Topical honey was shown to be effective in treating postoperative skin wounds in neonates that had failed to respond to antibiotic therapy.[4] It has been demonstrated in many studies that the antibacterial effects of honey are attributed to its high osmolarity, low pH, hydrogen peroxide content, and presence of other uncharacterized compounds.[5]

Royal jelly consists of an emulsion of proteins, carbohydrates, lipids, and other identified water-soluble compounds. Proteins make up about 13 percent of RJ, most of which belong to a family called major royal jelly proteins. The antibacterial activity of RJ has been described;[6] for instance, the royal jelly protein, royalisin, possesses antibiotic properties against gram-positive,

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but not gram-negative bacteria.[7] About 11 percent of RJ is sugars, including fructose and glucose, similar to those found in honey; lipids comprise about five percent of the substance.[8] The potency of antibacterial properties of RJ might be related to a particular fatty acid present in the ether-soluble fraction of RJ called trans-10-hydroxy decanoic acid (10-HDA).[6] The characterization of novel antibacterial peptides isolated from RJ, the jelleines, a series of short peptides presenting broad-spectrum activity against gram-positive bacteria, gram-negative bacteria, and yeasts, has recently been described.[9]

Honey mixed with other bee products is usually found in retail markets – honey with propolis and royal jelly being the most common. Due to the acidic taste of RJ, it is usually mixed with honey to increase consumer acceptance. The addition of honey also acts as a preservative to permit the storage of royal jelly at room temperature for an extended period of time.[10]

There is no known use of a honey-RJ mixture for therapeutic purposes, however. Therefore, the specific aim of this study is to evaluate, *in vitro*, the antibacterial properties of honey and RJ used together, information that could prove useful for practitioners wishing to use honey and/or RJ for management of superficial wounds. The correlation between the additive antimicrobial action of honey and RJ and the decrease in minimum inhibitory concentration (MIC) of both was statistically examined.

Material and Methods

Honey and RJ Samples

Four varieties of honey (V1 through V4) from different botanical origins (V1 and V2 were from orange blossoms and V3 and V4 were from eucalyptus) and one variety of RJ were analyzed. Samples were obtained directly from beekeepers in different regions of Algeria during 2006.

Bacterial Strain and Inoculum Standardization

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

Minimum Inhibitory Concentration Measurement

Concentrations of honey from 10-20 percent (volume/volume;v/v) and RJ from 1-5 percent (v/v) were incorporated into Mueller Hinton agar media to test efficiency against *P. aeruginosa*. The final volume of honey and media and RJ and media in each plate was 5 mL. The plates were inoculated and incubated at 37° C for 24 hours. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain would not grow.

In a second step, concentrations of honey less than the MIC were added to sub-MIC concentrations of RJ, which were then incorporated into media to determine the minimum additive inhibitory concentration against the *P. aeruginosa* strain. Similarly, the final volume in each plate was 5 mL. Plates were inoculated and incubated at 37°C for 24 hours and carried out in triplicate.

Statistical Analysis

Isobolographic analysis was carried out using Statistica® software to measure the additive antimicrobial action of honey and RJ against the tested bacteria.

Results

All varieties of honey and RJ were effective against *P. aeruginosa*. The effectiveness was related to the botanical origin of the honey. The MIC of the eucalyptus varieties (V3 and V4) was 12 percent (v/v) for both samples; whereas, the MIC of the orange varieties (V1 and V2) was 17 and 18 percent (v/v), respectively. The MIC of RJ was 4 percent (v/v), indicating RJ is more effective than honey against the tested strain of bacteria. Adding RJ to honey resulted in a significant decrease in the MIC of RJ and the honey varieties (Table 1; Figures 1 and 2). Each honey variety registered a greater than

Table 1. Combined Activity of RJ and the Four Varieties of Honey Against *P. aeruginosa*

RJ	0% RJ	1% RJ			2% RJ			3% RJ		
Honey varieties	Honey content in media	Honey content in media	Honey MIC drop	RJ MIC drop	Honey content in media	Honey MIC drop	RJ MIC drop	Honey content in media	Honey MIC drop	RJ MIC drop
V1	17% (v/v)	9% (v/v)	50%	75%	6% (v/v)	66.6%	50%	1% (v/v)	94.4%	25%
V2	18% (v/v)	9% (v/v)	50%	75%	6% (v/v)	66.6%	50%	1% (v/v)	94.4%	25%
V3	12% (v/v)	6% (v/v)	50%	75%	6% (v/v)	66.6%	50%	1% (v/v)	91.6%	25%
V4	12% (v/v)	6% (v/v)	50%	75%	6% (v/v)	66.6%	50%	1% (v/v)	91.6%	25%

Figure 1. Additive Activity of RJ and Honey V1 and V2 against *P. aeruginosa*

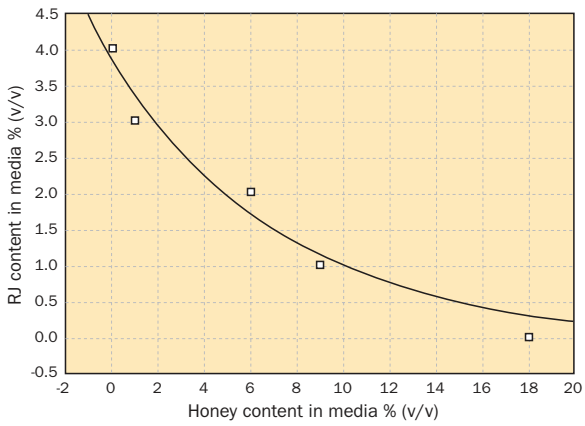


Figure 2. Additive Activity of RJ and Honey V3 and V4 against *P. aeruginosa*

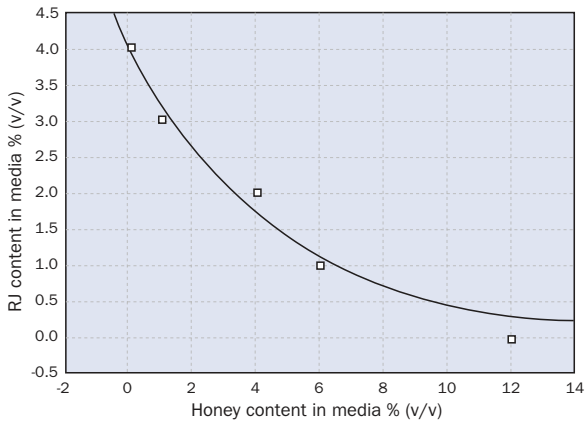


Figure 3. Negative Linear Regression Representing Correlation between the MIC Drop of RJ and Honey V1 and V2

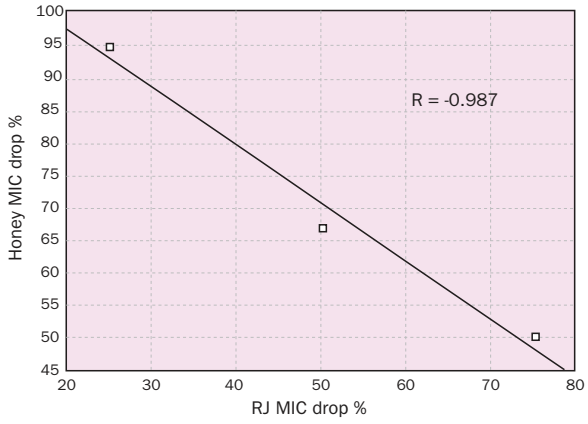
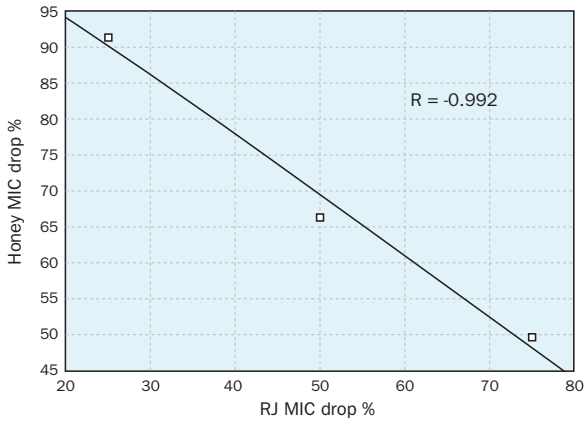


Figure 4. Negative Linear Regression Representing Correlation between the MIC Drop of RJ and Honey V3 and V4



90-percent decrease in MIC with 3-percent (v/v) RJ, a 66.6-percent decrease in MIC with 2-percent (v/v) RJ, and a 50-percent decrease in MIC with 1-percent (v/v) RJ. The MIC of RJ fell by 75 percent when used with half the concentration of honey that alone provides the MIC and by 50 percent when used with one-third the concentration of honey that alone provides the MIC. A strong linear correlation exists between the MIC drop of each variety of honey and RJ (Figures 3 and 4).

Discussion

Increasing interest is being accorded to the use of honey for managing wounds and burns.[11-14] Although honey offers broad-spectrum antimicrobial properties and promotes rapid wound healing,[15] the mechanisms by which these effects are achieved have not been fully elucidated. *P. aeruginosa* is the predominant cause of fatal burn wound sepsis,[1,2] and isolation of multi-drug 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 and RJ may receive renewed recognition as burn-wound healers. Both natural products are individually effective against bacteria, although joint use in wounds and burns has not been reported.

The results of this study show that adding honey to RJ increases the antibacterial effect against *P. aeruginosa*. Figures 1 and 2 demonstrate a synergistic effect of RJ and honey against the tested strain represented by isobolograms, which demonstrate several different dose combinations that attain the specified effect level.[16] Figures 3 and 4 show a strong linear correlation between the MIC drop of each variety of honey and RJ, expressed by negative linear regression (R); (R = -0.987 for V1 and V2; R = -0.992 for V3 and V4). Further research is needed to elucidate and optimize the effective combination of these two natural products in clinical practice. The current prevalence of antibiotic-resistant microbial species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey and RJ. Neither of these two natural products have an adverse effect on tissues, so they can safely be used on burns and inserted into cavities, including sinuses, to clear infection.

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