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RESEARCH ARTICLE

The effect of in vitro royal jelly provision on adhesion of *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic bacterium, which could aggressively infect immunocompromised patients and thus, cause high mortality rate. In addition, *P. aeruginosa* in oropharynx could be aspirated and cause ventilator associated pneumonia. Royal jelly is one of bee's products that has been used for therapeutic needs including antibacteria. Adherence factor of *P. aeruginosa* were flagelum, pili and lectin. The aim of the study was to determine the effect of royal jelly to *P. aeruginosa* adhesion. Suspension of *P. aeruginosa* (ATCC® 27853™) was incubated at 37 °C for 18 h. Treatment groups were exposed to royal jelly with several concentrations, 2%, 4%, 6%; while distilled water was being used as negative control. Bacterial adhesion test was determined using spectrophotometer λ = 600 nm to measure optical density values of adhered bacterial suspension in tubes. The result of one-way ANOVA showed significant differences ($p<0.05$) of optical density values among groups indicating that royal jelly affected the bacterial adhesion. LSD results showed significant difference of optical density values between 2%, 4%, and 6% royal jelly compared to distilled water. Six percent of royal jelly had the least optical density value compared to the other groups. In conclusion, royal jelly has the ability to decrease adhesion of *P. aeruginosa.* Six percent of royal jelly has better ability to decrease adhesion of *P. aeruginosa* than other concentrations.

Keywords: bacterial adhesion; *Pseudomonas aeruginosa*; royal jelly

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) can be found in the oral cavity of 5% of healthy individuals.[1] This bacterium may lead to infection in immunocompromised and vulnerable individuals as in cystic fibrosis sufferers or patients with burns.[2] *Pseudomonas aeruginosa* is responsible for approximately 58.8% of the death rate of patients due to septicemia in hospitals. Most bacterial transmission is associated with less precise sterilization of medical devices and nosocomial infections in hospitals.[3]

Pseudomonas aeruginosa may infect the oral cavity through food, drinks,[4] non-sterile mechanical ventilators,[3] dental unit water pipes, and patient suction devices.[5] The use of mechanical ventilators in hospitals can transmit nosocomial infections in the form of associated ventilators most pneumonia (VAP) that is mostly caused by *P. aeruginosa*.[6] Ventilators with less sterilization may contain bacteria, which colonize the oropharynx, are aspirated to the lungs, infect the lungs and cause pneumonia.[7] The bacteria can also contaminate daily dental practice.[8] In addition, bacteria may contaminate dental unit water from a water source to the dental unit and during the suction of the patient's saliva.[5]

Virulence factors of *P. aeruginosa* include exotoxin A and protease. Virulence factors that play a role in adhesion to target cells are also called adhesin, consisting of pili (fimbriae), *P. aeruginosa* lectins I (PA-IL), and *P. aeruginosa* lectins II (PA-IIL).[9] The ability of microorganisms to attach to the cell surface is an important factor in initiating pathogenesis activity.[10] Direct contact between infectious agents and host cells begins with an adhesion process (attachment). The adhesion process of *P. aeruginosa* starts from the initial interaction by pili bacteria binding to a specific series of carbohydrates from the glycoprotein membrane or the host cell glycolipid.[11]

The bacteria *P. aeruginosa* can attach to and form colonies in various cells such as buccal,

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lung, kidney and endothelial cell epithelium.[12] The attachment of *P. aeruginosa* to buccal epithelial cells is influenced by levels of fibronectin on the surface of host cells and salivary proteases. Immunocompromised patients have higher protease levels than normal individuals. Increased salivary protease levels cause a decrease in fibronectin levels. This results in *P. aeruginosa* being more easily attached to buccal cells of immunocompromised patients.[13]

Honey and royal jelly have the potential for treating bacterial infections that are getting more resistant to antibiotics. Honey is proven effective in killing biofilm isolates of *P. aeruginosa* and *Staphylococcus aureus* that are resistant to antibiotics in patients with chronic rhinosinusitis.[14] According to Boukraa,[15] all types of honey and royal jelly effectively inhibit *P. aeruginosa.* The minimum inhibitory concentration (MIC) of 4% royal jelly indicates that royal jelly is more effective at inhibiting the growth of *P. aeruginosa* than honey, which has various MIC of 12% -18% based on each different type of honey.

One of the focuses of the study of antibacterial materials is to prevent microbial adhesion to host cells since the beginning of infection.[9] Rachmaninov et al.[16] used bird eggs, royal jelly, fruits and seeds to inhibit the attachment of lectin PA-IL and PA-IIL. Royal jelly is known to be able to bind to PA-IL and PA-IIL found in *P. aeruginosa* during the hemagglutination process, but how it affects the bacterial adherence itself remains unknown. This study aims to determine the effect of royal jelly on the adhesion ability of *P. aeruginosa* bacteria.

MATERIALS AND METHODS

This research has been declared ethically feasible by the ethics and advocacy unit of the Faculty of Dentistry, Universitas Gadjah Mada. Ethical Clearance Number 0026/KKEP/FKG-UGM/EC/2014. Royal jelly-producing bees are obtained from beekeepers in Nusukan, Surakarta, Central Java. Bees are sent to the Animal Species Analysis Division, Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada, to determine the species. The test revealed that the bees were from the species of *Apis mellifera.*

Royal jelly was dissolved in distilled water to obtain a concentration of 2%, 4%, 6%. *Pseudomonas aeruginosa* was cultured in BHI broth and incubated at 37 °C for 24 hours. Bacteria were harvested by centrifuging 10,000 rpm for 10 minutes and washed twice with 1x PBS. Bacterial suspension were homogenized using vortex and then adjusted to 0.5 McFarland standard.

In the treatment group, 1.5 ml of royal jelly with an initial concentration of 2%, 4%, and 6% was added to the test tube containing 3 ml of BHI broth. Then, 0.1 ml of the suspension of *P. aeruginosa* (1.5×10^8 CFU/mL) was added to equalize the number of bacteria in each sample. The control group was added with distiled water. The tubes were incubated at 37 °C for 18 hours in a tilted position of 30°. The supernatant of the culture in the tube was then discarded, and the tube was rinsed with 5 ml of distilled water. The bacteria attached to the tube wall were released from the culture tube using 2 ml of 0.5 M sodium hydoxide into the tube and being vortexed. The bacterial suspension was transferred into the cuvette for optical density measurements using a spectrophotometer at a wavelength of 600 nm. All treatment groups were made in 6 replications. The optical density readings on the spectrophotometer (λ 600 nm) were conducted 3 times for each treatment and then averaged.

RESULTS

The results showed that optical density in the group provided with royal jelly was lower than that of the negative control. The higher the concentration of royal jelly in the test group the lower the optical density value (Figure 1). Low optical density values indicate a low ability of bacteria to attach to the walls of glass tubes.

The data normality was then tested with Shapiro-Wilk and its homogeneity was tested using Levene's test. The Shapiro-Wilk test showed results that were greater than 0.05 ($p>0.05$) indicating the normal data distribution. The Levene's test showed a significance value of 0.013 ($p<0.05$) indicating that the data on the research results were not homogeneous. Therefore, the transformation was done using MiniTab. Afterwards, the results of the

data transformation were then tested again with Shapiro-Wilk and Levene's test.

The Shapiro-Wilk test results showed that the value of all treatment groups were more than 0.05 (p>0.05), which means that the data were normally distributed. Furthermore, the homogeneity test showed a significance value of 0.544 indicating that the data were homogeneous.

One-way ANOVA analysis showed p = 0.000 (p<0.05). These results indicate that royal jelly significantly influences the adhesion ability of *P. aeruginosa* bacteria. The post hoc test in the form of an LSD test in Table 1 was conducted to determine the comparison of significance between treatments.

The LSD results showed significant differences between all treatment groups compared to control and 4% royal jelly or 6% royal jelly. Significant differences were detected between the 2% royal jelly group and the 4%. These results indicate that all royal jelly concentrations used in this study have the ability to reduce the attachment of *P. aeruginosa* bacteria to the surface of glass tubes.

Table 1. LSD analysis comparing the significance of the optical density value of *P. aeruginosa* between treatments

	2% Royal jelly	4% Royal jelly 4%	4% Royal jelly 6%
2% Royal jelly	-	0.236	0.000*
4% Royal jelly	0.236	-	0.000*
6% Royal jelly	0.000*	0.000*	-
Aquadest	0.000*	0.000*	0.000*

*The value of p<0.05 = there are significant differences

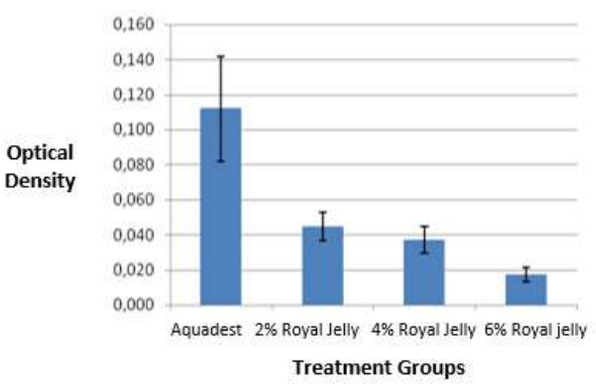

Figure 1. Graph of optical density mean and standard deviation of the test group and negative control group. The value of optical density decreases along with the increasing concentration.

DISCUSSION

In vitro bacterial attachment was measured by comparing the bacterial optical density attached to the test tube during the reading process using spectrophotometer with a certain wavelength. The higher optical density obtained from the spectrophotometer indicates the increasing number of bacteria attached to the test tube surface.[17] The study notes that the attachment ability of *P. aeruginosa* bacteria decreases after the administration of royal jelly. The higher the concentration of royal jelly, the lower the optical density, which means that fewer bacteria can attach to the tube wall.

Royal jelly, which is white to yellowish,[18] make optical density readings less accurate. This can be overcome by rinsing the tube with distilled water. Aquadest are only flowed through the tube walls so as not to disturb the bacteria attached to the tube wall. The research method section explained that each sample (a culture tube containing 3 ml BHI and 1.5 ml royal jelly or aquadest) was inoculated with 0.1 ml bacterial suspension (1.5 x 10^8 CFU/ml) to make even the number of bacteria in each sample. This process is to ensure that the bacteria attached to the tube wall (biofilm) are bacteria that survive rom the antibacterial properties of royal jelly.

Pseudomonas aeruginosa has pili containing lectins namely PA-IL and PA-IIL, which play a role in adhesion.[9] The reduction in bacterial adhesion after administration of royal jelly is possible because royal jelly is able to block the lectin by providing receptors resembling decoys. As a result, lectin can no longer bind to host cell receptors if it has been linked to glycodecoys first.[16] Royal jelly contains a complex mixture of proteins, sugars, fats, some minerals, and vitamins.[18] However, active substances in royal jelly that specifically act as glicodecoys are still unknown.

A decrease in bacterial adhesion ability may also be attributed to the antibacterial ability of royal jelly. The 4% royal jelly is known to be able to inhibit the growth of *P. aeruginosa*.[15] The royal jelly content identified as antibacterial includes 10-hydroxy-trans-(2)-decanoic acid (HDA), royalisin, apimicin, jellenies I, II, III, IV and apalbumina α.[19]

This finding is in line with the previous research revealing that 4% royal jelly can inhibit the growth of *P. aeruginosa*.[15] The possibility of glycodecoys as an anti-adhesion and antibacterial content in royal jelly is mutually supportive, so the optical density values of samples given royal jelly were lower than that was of aquadest group.

In this study, royal jelly with concentrations below 4% or 2% indicated the ability to inhibit the adhesion of *P. aeruginosa* with a significant difference compared to the negative control. Significant differences can also be observed in the group treated with 4% and 6% royal jelly. However, the group treated with 2% and 4% royal jelly showed insignificant differences of optical density, because the royal jelly active substances having the potential to inhibit the attachment of *P. aeruginosa* was not as much as 6%. The 6% royal Jelly has the best ability to inhibit bacterial attachment because it contains higher concentrations of glycodecoys and higher antibacterial content. This study has not found the optimum concentration to inhibit the attachment of *P. aeruginosa.*

CONCLUSION

Based on the results of the study, it was concluded that royal jelly can reduce the attachment of *P. aeruginosa.* Concentration of 6% royal jelly has the best ability to reduce bacterial attachment compared to other tested concentrations.

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