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Propolis-Sahara honeys preparation exhibits antibacterial and anti-biofilm activity against bacterial biofilms formed on urinary catheters

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ABSTRACT

Objective: To evaluate the antibacterial effect of Sahara honeys (SHs) against bacterial biofilms formed on urinary catheters in combination with propolis-Sahara honeys (P-SHs).

Methods: Three clinical isolates were subjected to biofilm detection methods. The antibacterial and anti-biofilm activity for SHs and P-SHs were determined using agar well diffusion and the percentage of biofilm inhibition (PBI) methods.

Results: The PBI for Gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*)] was in the range of 0%–20%, while PBI for Gram-negative bacteria [*Pseudomonas aeruginosa* and *Escherichia coli (E. coli)*] were in range of 17%–57% and 16%–65%, respectively. The highest PBI (65%) was produced by SH2 only on *E. coli*. In agar well diffusion assay, zones of inhibition ranged from 11–20 mm (*S. aureus)*, 9–19 mm (*Pseudomonas aeruginosa)* and 11–19 mm (*E. coli)*. The highest inhibition (20 mm) was produced by SH1 only on *S. aureus*. In addition, the treatment of SHs and P-SHs catheters with a polymicrobial biofilms reduced biofilm formation after 48 h exposure period.

Conclussions: SHs and P-SHs applied as a natural agent can be used as a prophylactic agent to prevent the formation of *in vitro* biofilm.

1. Introduction

A number of previous studies have shown the urinary tract colonization and infection caused by *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) in patients with indwelling urinary catheters[1,2]. Al-Mathkhury *et al.*[3] demonstrated the Gram-negative opportunistic *P. aeruginosa* common colonization of urinary catheters and biofilm development on them. Several factors may contribute for the pathogenicity of bacterial biofilm formation, including the production of extracellular compounds (*E. coli*: flagellum; *S. aureus*: lipopolysaccharides, exopolysaccharide; *P. aeruginosa*: flagella and pili), production of resistant "persister cells", surface adherence and biofilm formation[4-6]. The adhesion of bacteria to a surface depends on various factors (nutrient levels, pH changes, desiccation, ultraviolet radiation and osmotic stress)[7,8]. More recently, some substances showing antibacterial properties, such as nitrous oxide chlorhexidine, nitrofurazone and gentian violet, have been used to modify the surface of urinary catheters[9]. But the biofilms are notoriously difficult to eradicate. In addition to the difficulty of treating biofilms with conventional antibiotics, recently alternative treatments are playing their role in the treatment of biofilms.

The antimicrobial activities of bee products, such as honey and propolis, have been researched over recent years as alternatives for new therapeutic agents for the treatment of bacterial biofilm infections[10,11]. Algerian honey [Sahara honey (SH)] was reported to inhibit the growth of *S. aureus*, *P. aeruginosa* and *E. coli*[12,13].

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Today, no information is available about the effects of SHs on biofilms. Therefore, this study was performed to investigate the role of SHs at different concentrations alone or in combination with propolis-Sahara honeys (P-SHs) on biofilms. We also investigated the effects of P-SHs on biofilms for the first time.

2. Materials and methods

2.1. Honey and propolis samples

The present study was carried out on raw SH of different floral origins, namely, *Euphorbe* (SH1) (*Euphorbia* spp.) and Sidr honey (SH2). The propolis used in this study was obtained from Southern Algeria.

2.2. Preparation of propolis solutions

The propolis was cold-macerated to make an extract with olive oil (20 g of brute propolis/2 mL of olive oil). The mixture was heated at 50 ˚C for 15 min before microbiological testing.

2.3. Preparation of honey with olive oil – propolis

The mixture was stirred gently with a spatula until homogeneous gel was formed. The mixture was heated at 50 ˚C for 15 min. For a microbiological test of a mixture of honey, 20 g of propolis was made, where the honey was added in a concentration of 25%, 50% or 100%.

2.4. Bacterial isolates and growth media

The catheters were removed from patients and then cut under aseptic conditions using a sterile scalpel. The catheter was carefully and aseptically cut. Three discs were placed on the surface of Chapman, MacConkey and King A agar plates. Colony formation was monitored by examining plates after 48 h of incubation.

2.5. Antibacterial susceptibility testing

In this study, two different assays were performed to evaluate the antibacterial potential of the honey samples: agar-well diffusion (AWD) and percentage of biofilm inhibition (PBI).

2.5.1. AWD

Antibacterial studies have been evaluated by the method of AWD by Moussa *et al.*[13]. Briefly, agar plates (90 mm) were containing 20 mL of nutrient agar at 37 ˚C for 24 h and adjusted by diluting fresh cultures to a turbidity equivalent to 0.5 McFarland scale (approximately 2×10^8 colony-forming unit/mL). An 8 mm diameter well was cut into the agar and 100 μL of undiluted, and 25% and 50% honey solution (w/v) prepared in sterile distilled water was aliquoted into the well. The controls were set up with equivalent quantities of water. After incubation, the diameters of the inhibition zones were measured.

2.5.2. PBI

The method adopted was described by Akujobi and Njoku with little modification[14]. Briefly, 0.2 mL of 0.5 McFarland standardised culture was added to 4 mL of the test (SHs and P-SHs). Concentration in a test tube while inoculation of 4 mL of nutrient broth with 0.2 mL of the cell suspension was served as the control. The optical density (OD) was determined in a spectrophotometer at 620 nm prior to incubation (T0) and recorded after the cultures were incubated for 24 h in the dark at 37 ˚C. The OD was determined at T0 and again after 24 h of incubation at 620 nm. The OD for each replicate at T0 was subtracted from the OD for each replicate after 24 h of incubation. The PBI was calculated using the following formula:

PBI% = [(OD control – OD experimental)/OD control] × 100

OD = absorbance at 620 nm.

2.5.3. Biofilm response to SHs and P-SHs

The bacterial anti-adhesive activity of the SHs and P-SHs against bacterial biofilms was qualitatively evaluated by the following method (Table 1).

Table 1

Exposure of SHs and P-SHs treatment on bacterial biofilm.

Tube	Experiment I	Treatment after 24 h	Experiment II	Incubation
Tube 1	Nutrient broth + catheter	Negative control	Nutrient broth + catheter bacterial (single and mixed)	48 h
Tube 2	Nutrient broth + catheter	SHs (25%, 50% and 100%)	Nutrient broth + catheter bacterial (single and mixed)	48 h
Tube 3	Nutrient broth + catheter	Propolis	Nutrient broth + catheter bacterial (single and mixed)	48 h
Tube 4	Nutrient broth + catheter	P-SHs (25%, 50% and 100%)	Nutrient broth + catheter bacterial (single and mixed)	48 h

Tube 1: Sterile catheter segments were immersed in 5 mL sterile culture tubes nutrient broth and incubated at 37 ˚C for 24 h (Experiment I), and after 24 h, the tubes were inoculated with 100 μL of bacterial inoculum (2×10^8 cells/mL) and incubated at 37 ˚C for 48 h (Experiment II); Tube 2: Sterile catheter segments were immersed in 5 mL sterile culture tubes nutrient broth + SHs (25%, 50% and 100%) and incubated at 37 ˚C for 24 h (Experiment I), and after 24 h, the tubes were inoculated with 100 μL of bacterial inoculum (2×10^8 cells/mL) and incubated at 37 ˚C for 48 h (Experiment II); Tube 3: Sterile catheter segments were immersed in 5 mL sterile culture tubes nutrient broth + propolis and incubated at 37 ˚C for 24 h (Experiment I), and after 24 h, the tubes were inoculated with 100 μL of bacterial inoculum (2×10^8 cells/mL) and incubated at 37 ˚C for 48 h (Experiment II); Tube 4: Sterile catheter segments were immersed in 5 mL sterile culture tubes nutrient broth + P-SHs at (25%, 50% and 100%) and incubated at 37 ˚C for 24 h (Experiment I), and after 24 h, the tubes were inoculated with 100 μL of bacterial inoculum (2×10^8 cells/mL) and incubated at 37 ˚C for 48 h (Experiment II).