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FTIR characterization of Sahara honey and propolis and evaluation of its anticandidal potentials

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Abstract: *Antifungal of bee products have been shown to be protective against microbial biofilms invasion Sahara honey and propolis were applied as an antimicrobial in treatment for many infections in Algeria. In this study, Fourier transform infrared spectroscopy (FTIR) analysis was utilized to characterize the chemical structures and functional groups. In addition, in this study, we determined the anti-candida activity of honey used alone or in combination with propolis. Proteins, carbohydrates, carboxylic acids, free amino acids, cellulose and lipids, ketones and phenol compounds were identified by FTIR analysis. Combination of Sahara honey and propolis increased antifungal efficacy, compared to compounds tested alone. Propolis increased the anti-candidal effect of Sahara honey. In addition, the treatment of Sahara honey and Propolis-Sahara honey catheters with a Candida albicans biofilms reduced biofilm formation after 24 and 48-h exposure period. The results provide evidence that honey/propolis combination may help in designing a more potent novel, natural antibiofilm blend at sufficiently low concentrations in medical domain.*

Keywords: *FTIR, Candida albicans, Honey, Propolis, Antibiofilm activity*

Introduction

There are different types of antimicrobial agents which inhibit the colonization of the catheters with pathogenic microbes [1]. Many studies report the efficacy, advantages and disadvantages regarding the use of antimicrobial agents. Several studies have been conducted to evaluate the effectiveness of antiseptic catheters in preventing catheter-associated urinary tract infection [2]. According by Danese [3] a number of factors conspire to render catheter implants especially susceptible to microbial contamination. In addition, catheters

can also be contaminated in their luminal compartments where fluid flow from contaminated infusate solutions can provide microbial pathogens rapid access to the vasculature [4].

According to the recommendations of several researchers [5-6] the simplest method for preventing bacterial colonization and eventual biofilm formation on catheters is to impregnate the catheter itself with a broad-spectrum antimicrobial agent that elutes from the device and impairs bacterial growth through traditional bactericidal or bacteriostatic mechanisms.

Many bee products have antimicrobial and antibiofilm effects *in vitro*. They inhibit not only the formation of biofilms but also the quorum sensing system. 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 yeast infection [7-8].

Multiple properties of honey and propolis like antimicrobial and anti-biofilm have been proven in many studies. In 2017 in a study conducted by Aissat et al. [9] two different pathogenic isolates were compared for the efficacy of Sahara honey and propolis. These bee products did not present antagonistic interactions between them, allowing their combined use, which can contribute to an increase in their effects against biofilms.

But there is no study that evaluates the anti-candidal activity of propolis and honey against *Candida albicans* biofilm formed on urinary catheters. In this investigation, we evaluated the anti-candidal activity of propolis and honey against *C. albicans*. Also this study evaluated the enhances the antiadhesive activity and anti-biofilm potential of Sahara honey by propolis against these clinical isolate to the same protocol according to Aissat et al. [9] with complementarity by characterization by Fourier Transform Infra-Red spectroscopy to identify the structure and chemical groups of the products.

Materials and methods

Honey and propolis samples

The present study was carried out on raw Sahara honey namely Euphorbe (*Euphorbia spp.*). The propolis used in this study was obtained from southern Algeria.

Fourier Transform Infra-Red Spectroscopy Profile

Functional groups and types of chemical bonds present in phytochemicals are identified by Fourier transform infrared spectroscopy analysis. All the experimental studies have been according by Aissat et al. [9].

Preparation of propolis solution

The propolis was cold-macerated to make an extract with olive oil (brute propolis 20 g / olive oil 2 mL). The mixture was heated at 50°C for 15 min before microbiological testing. The solution was stored at room temperature throughout the experiment and protected from light.

Preparation of honey solutions

Honey solutions were prepared immediately prior to testing by diluting honey to the required concentrations (undiluted (100%), 25% and 50% w/v). All samples were then incubated for 30 min at 37°C in a shaking water bath that allowed aeration of the solutions. Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive [10].

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. With continued mixing for 15 minutes. For a microbiological test of a mixture of honey and propolis 20 g was made, wherein the honey is added in a concentration of 25%, 50% or 100%.

Yeast strain and culture conditions

Yeasts were maintained on Sabouraud dextrose agar (SDA; BioMérieux, Marcy l'Etoile, France) at 4°C, and sub cultures were performed prior to each experiment in the same medium for 48 h at 35°C. Turbidity standard and preparation of inocula: stock fungal inoculum suspensions were prepared in sterile saline from 48 h cultures on SDA at 35°C. Each suspension was adjusted visually to 0.5 McFarland turbidity standards. Dilutions of these suspensions were subcultured on SDA to determine the number of CFU/mL. The adjusted inoculum was (approximately 2×10^8 CFU/mL) [11].

In this study two different assays were performed to evaluate the anticandidal potential of the tested products: agar-well diffusion and percentage of biofilm inhibition.

Agar well diffusion method

Agar-well diffusion assay was used to evaluate the antifungal activity of the SHs and P-SHs based on Ahmed et al. method [11]. Briefly, agar plates (90 mm) were containing 20 mL of Sabouraud dextrose agar at 37°C for 24 h and adjusted by diluting fresh cultures to turbidity equivalent to 0.5 McFarland scale (approximately 2×10^8 CFU/mL). An 8-mm diameter well was cut into the agar and 100 µL of undiluted, 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 as controls. All plates were incubated overnight at 35°C, and the zone of inhibition was measured after 28 h of incubation.

Percentage of biofilm inhibition

The Percentage of biofilm inhibition percentage of biofilm was calculated using the following formula [12]:

$$\text{Percentage of biofilm inhibition \%} = [(\text{OD}_{\text{control}} - \text{OD}_{\text{experimental}}) / \text{OD}_{\text{control}}] \times 100,$$

Where OD = absorbance at 620 nm

Inhibition and eradication of catheter biofilms of the Sahara honey and Propolis-Sahara honey

The anti-adhesion activity of Sahara honey and Propolis-Sahara honey against *C. albicans* biofilms was performed in experiments before and after-incubation.

Experiments I

Tube 1: Tube containing only the Sabouraud dextrose broth and sterile catheter, but without microorganisms, were used as controls.

Tube 2: Tube containing only the Sabouraud dextrose broth and sterile catheter and Sahara honey (25, 50 and 100%) was added to each tube and all tubes were incubated at 35°C for 24 h and 48 h.

Tube 3: Tube containing only the Sabouraud dextrose broth and sterile catheter and propolis was added to each tube and all tubes were incubated at 35°C for 24 h and 48 h.

Tube 4: Tube containing only the Sabouraud dextrose broth and sterile catheter and propolis and Sahara honey (25, 50 and 100%) was added to each tube and all tubes were incubated at 35°C for 24 h and 48 h.

Experiments II

After 24 h and 48h, 100 microliter of the yeast suspension was added to each tube and all tubes (1, 2, 3 and 4) were incubated at 35°C for 24 h and 48 h.

Results and Discussion

FTIR spectroscopy was used to Sahara honey s based on their spectral differences in the 4000 cm^{-1} to 500 cm^{-1} spectral region. A representative FTIR spectrum of SH is shown in Figure 1 and Table 1.

The absorption bands between 3700 and 3000 cm^{-1} are due to stretching vibrations of the functional group -OH from carbohydrates, water, and organic acids presents in the honey. The absorption bands between 2931 and 2931 cm^{-1} are due to stretching vibrations of the functional group -OH from carbohydrates, proteins carboxylic acids, free amino acids, cellulose and lipids, ketones and phenol and water presents in the SH and PSH (Figure 2 and Table 2). According to some authors, peak at 918 cm^{-1} and 2931 cm^{-1} corresponds to the C–H bending of the carbohydrate, C–O stretch in the C–OH group as well as the C–C stretch in the carbohydrate structure, respectively [13-25].

Table 1. General band assignment of FTIR spectrum of SH

Wavenumber cm^{-1}	Vibrations
3346	C-H stretching from proteins
2848 - 2915	C-H stretching (carbohydrates) O-H stretching (carboxylic acids) NH ₃ stretching (free amino acids) C-H stretching bending of cellulose and lipids
1736	free acids
1644- 1696	O-H stretching/bending (water) C=O stretching (mainly from carbohydrates) N-H bending of amide I (mainly proteins)
1168- 1462	O-H stretching/bending C-O stretching (carbohydrates) C-H stretching (carbohydrates) C=O stretching of ketones
1168–919	C-O & C-C stretching (carbohydrates) Ring vibrations (mainly from carbohydrates)
919–719	Anomeric region of carbohydrates C-H bending (mainly from carbohydrates)

Figure 1: Average FTIR spectrum of the Sahara honey.

Table 2. General band assignment of FTIR spectrum of Propolis-Sahara honey

Wavenumber cm^{-1}	Vibrations
3346	C-H stretching from proteins
2848 - 2915	C-H stretching (carbohydrates) O-H stretching (carboxylic acids) NH ₃ stretching (free amino acids) C-H stretching bending of cellulose and lipids
1736	free acids
1644- 1696	O-H stretching/bending (water) C=O stretching (mainly from carbohydrates) N-H bending of amide I (mainly proteins)
1168- 1462	O-H stretching/bending C-O stretching (carbohydrates) C-H stretching (carbohydrates) C=O stretching of ketones
1168–919	C-O & C-C stretching (carbohydrates) Ring vibrations (mainly from carbohydrates)
919–719	Anomeric region of carbohydrates C-H bending (mainly from carbohydrates)

Figure 2: Average FTIR spectrum of the PSH.

The anticandidal effect of the combination of Sahara honey at different concentrations (25% (w/v), 50% (w/v) and 100 % (w/v) with propolis on *C. albicans* was monitored at 48 h.

Figure 3 shows the diameters of the zones of inhibition of Sahara honey against *C. albicans* determined by well diffusion assay. The results showed that the diameters of the inhibition zones for *C. albicans* were 8–10 mm. The combinations of Sahara honey and propolis showed the highest synergistic activity against *C. albicans* when combined at concentrations of 100% and 50%. The combinations that caused relatively large zones of inhibition (12 mm) are shown in Figure 3.

The percentage of biofilm inhibition of the Sahara honey and propolis-Sahara honey, were observed in 24 h incubated. Almost 33.57% of biofilm inhibition was observed at 25% of Sahara honey. The anticandidal activity of Sahara honey and propolis were synergistic with a concentration 50% (98.92%) are shown in Figure 4.

In a second series of experiments, the disruption of preformed biofilms (24 h and 48 h growth in the absence of Sahara honey, Propolis and propolis-Sahara honey by addition of Sahara honey, Propolis and propolis-Sahara honey at various concentrations for 24 h and 48 h the Sahara honey, Propolis and propolis-Sahara honey inhibited biofilm formation on catheters (Table 3). This suggests that Sahara honey, Propolis and propolis-Sahara honey have a better ability prevented biofilm formation.

Figure 3. Mean Zones of Inhibition values of the Sahara honey and Sahara honey-propolis against *C. albicans*

Figure 4. Growth inhibitory activity of the Sahara honey and propolis-Sahara honey against *C. albicans*

Table 3. Effects of Sahara honey, Propolis and Sahara honey-propolis on the *C. albicans* Growth and Biofilm Formation

Adhesion assay test	After incubation 24h	After incubation 48h
Sabouraud dextrose broth +catheter (Negative control)	BF (++)	BF (++)
Sabouraud dextrose broth + catheter + Sahara honey	BF (--)	BF (--)
Sabouraud dextrose broth +catheter + Propolis	BF (--)	BF (--)
Sabouraud dextrose broth + catheter + Propolis-Sahara honey	BF (--)	BF (--)

BM: Biofilm Formation; BF++(adhesion); BF— (no adhesion)

C. albicans is a facultative pathogenic microorganism that has developed several virulence factors that contribute to pathogenesis (membrane and cell wall barriers, dimorphism, biofilm formation, signal transduction pathway, proteins related to stress tolerance, hydrolytic enzymes and toxin production [26]. Therefore, *C. albicans* biofilm formation is considered an important matter because it can lead to strong resistance to conventional antifungal agents [27].

In addition, the conventional antifungal agents are not only harmful to the human when used at high concentrations but also inefficient in suppressing biofilm of *C. albicans* [28]. Therefore, many researchers are

focusing on searching for new compounds with low toxicity that can effectively inhibit biofilm formation in *C. albicans*.

Recent evidence indicates that bee products (honey and propolis) have potential therapeutic benefits in several contexts, including microbial infection (biofilms). In addition, it was reported that honey and propolis have shown a broad spectrum of antimicrobial activity against fungi and bacteria in disease infection. Potent novel antimicrobials are also of interest to treat biofilms. Several studies have also shown an important activity of honey and propolis against microbial biofilms.

Propolis is composed of more than 300 different components, like polyphenol (Flavonoids, diterpenic acids, aromatic acids and phenolic aldehydes, pinocembrin, apigenin and ketones) as the principal components that are responsible for the biological activities of propolis samples [29]. In addition, the antimicrobial nature of honey depends on different factors acting singularly or synergistically, the most salient of which are various phenolic compounds, complex carbohydrates, wound pH, H₂O₂, pH of honey and osmotic pressure exerted by the honey itself [30-31]. Also, previous studies have demonstrated the ability of two flavonoids of the honey (chrysin and galangin) to cross the membrane of *C. albicans* altering the normal fungal metabolism [32].

To our knowledge, this is the first report on the anti-biofilm activity of Sahara honey and the synergistic effects of the mixture of Sahara honey-propolis against *C. albicans* biofilm formation.

Results of preliminary anti-candidal tests, performed by the Agar well diffusion and percentage of biofilm inhibition method, were quite variable between Sahara honey and Sahara honey-propolis. In addition, Sahara honey and Sahara honey-propolis exhibited excellent anti-adhesive properties against *C. albicans* as evidenced in our adhesion based assays. The possible reason for these variations between bacteria response to propolis-Sahara honey might be due to difference in their chemical composition (flavonoids) botanical sources, and the season of collection.

Conclusion

In conclusion, our results suggest that therapeutic concentrations of Sahara honey and propolis display potent *in vitro* activity against *C. albicans* biofilms. These results are in agreement with those presented in a recent report by Aissat and colleagues, who also demonstrated potent *in vitro* activity by Sahara honey and propolis against preformed biofilms of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. These data indicate that a Sahara honey in combination with propolis can act as promising natural lubricant for preventing catheter-associated urinary tract infections.

Conflicts of interest

No potential conflicts of interest have been declared.

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