

Antifungal effect of honey against *Candida albicans* isolated from saliva

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ABSTRACT

The antifungal activity of different concentration of natural honey , synthetic honey and sugar syrup were examined against ten strains *Candida albicans* that isolated from saliva of patients with dental caries and gingival ulcer . The antifungal test was done by agar dilution method by adding honey to the agar after sterilization . The concentrations used in this study of all sample were ranged from (10%-85%) (w/v) The natural honey MIC that inhibited *C. albicans* growth ranged from (20%-40%)(w/v%) . A synthetic honey and sugar solution was also tested to determine antifungal activity attributable to sugars. *C. albicans* was more resistant against the presence of synthetic honey and sugar syrup in the culture medium at concentrations above 70% (w/v%) . What the data suggests is that the component in the honey responsible for the observed antifungal in vitro properties is not sugar based. Honey might be tapped as a natural resource to look for new medicines for the treatment of mycotic infections. This could be very useful, onside ring the increasing resistance of antifungal.

Introduction

The majority of human mycoses are caused by opportunistic fungi (1). *C. albicans* is a fungus which can grow on warm and moist surfaces and cause superficial diseases such as oral and vaginal thrush and chronic mucocutaneous candidiasis (2).

Honey is a mix of natural sugars (80%), water (18%) and minerals, vitamins, pollen, protein and amino acids (2%). Around 70% of honey's natural sugar content is made up of fructose and glucose (3) . Raw honey is anti-bacterial, anti-viral, anti-fungal, and an antioxidant. It has been shown to inhibit the growth of pathogens in food and food spoilage organisms(4).

Honey is a natural product that is used for its antifungal activity (5). Its antimicrobial properties have been extensively reviewed (6). Honey has a potent antibacterial ctivity and is very effective in clearing infection in wounds and protecting them from becoming infected (7). Honey is particularly effective as an antibacterial agent against *Staphylococcus aureus*, *E. coli* and *Candida albicans* (5,8) .

Materials and Methods

Honey samples and sugar syrup

Natural honey was obtained from(Iraqi apiary) .Synthetic honey was obtained from the market and the sugar syrup prepared in the laboratory by dissolved 180 gm sugar in 20 ml sterile distal water (concentration of stock solution 90% (w/v%)) and different concentrations of syrup (10-90% (v/v%)) were prepared from stock solution . A stock solution of natural honey were prepared by dissolving 120gm of natural honey in 80 ml sterile distal water (60% (w/v%)) and different concentration of natural honey (10-60% (v/v%)) were prepared . In the same way the concentration of synthetic honey were prepared by dissolving 170 gm honey in 30 ml sterile D.W(85% (w/v%)) .All these concentrations of natural , synthetic honey and sugar syrup were filtered and added to the Muller-Hinton agar to test the antifungal effected .

Fungal strain and inoculums standardization

Ten *Candida albicans* strains have been isolated from saliva samples from patients with dental caries and gingival ulcers .All samples were culture in Sabouraud dextrose agar . All strains were diagnosis according to the germ tube test (9) It was maintained by subculture in sabouraud dextrose agar media . The inoculums suspensions of *C. albicans* was obtained by

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taking five colonies from 24-h-old cultures grown on subouraud dextrose media . The colonies were suspended in 5 mL of sterile saline (0.85% NaCl). The inoculum suspensions were shaken for 15 s and the density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to $1-5 \times 10^6$ cfu/mL) with sterile saline. The suspensions were diluted 1:1000 to give a final inoculums suspension equivalent to $0.5 - 2.5 \times 10^3$ cfu/mL (NCCLS)

Minimum inhibitory concentration (MIC) of Natural honey

Concentrations of natural honey between 10% to 60% (v/v%) were incorporated into Muller- Hinton agar media after sterilization to test their efficiency against *C. albicans*. The final volume of honey and media in each plate was 12.5 mL. The plates were inoculated and incubated at 37°C for 48 h. The MIC (Minimum Inhibitory Concentration) 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 (v/v%).

Minimum inhibitory concentration (MIC) of synthetic honey and sugar syrup

Concentrations of synthetic honey and sugar syrup between 10% and 90% (v/v%) were incorporated into Muller –Hinton agar media after sterilization to test their efficiency against *C. albicans*. The final volume of solutions and agar media in each plate was 12.5 mL. The plates were inoculated and incubated at 37°C for 48 h . The MIC (Minimum Inhibitory concentration) was determined by finding the plates with the lowest concentration of honey or sugar syrup on which the strain would not grow. All MIC values are expressed in (v/v%). All inoculations were carried out in duplicates.

Results

Antifungal effects of natural ,synthetic honey and sugar syrup were tested against ten *Candida albicans* strains that isolated from saliva .The results show that the MIC of Natural honey range from 20%-40% (v/v%) (Table 1) .

Table 1. Numbers of inhibited *Candida albicans* strains by different concentrations of natural honey on Muller-Hinton agar plates after 48hrs incubation at 37°C .

Concentrations of natural honey (v/v%)	Number of inhibited <i>C.albicans</i> strains	%of inhibited <i>C.albicans</i> strains
10	0	0%
20	7	70%
30	8	80%
40	10	100%
50	10	100%
60	10	100%

While effected concentration of synthetic honey and sugar syrup range from 60%-85% (v/v%) (Table 2,3) .

Table 2. Numbers of inhibited *Candida albicans* strains by different concentrations of synthetic honey on Muller-Hinton agar plates after 48hrs incubation at 37°C .

Concentrations of synthetic honey (v/v%)	Number of inhibited <i>C.albicans</i> strains	%of inhibited <i>C.albicans</i> strains
10- 60	0	0%
70	6	60%
80	8	80%
85	10	85%

Table 1. Numbers of inhibited *Candida albicans* strains by different concentrations of sugar syrup on Muller-Hinton agar plates after 48hrs incubation at 37°C .

Concentrations of sugar syrup (v/v%)	Number of inhibited <i>C.albicans</i> strains	%of inhibited <i>C.albicans</i> strains
10- 60	0	0%
70	5	50%
80	8	70%
85	10	85%

Discussion

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, uncharacterized compounds (10).

In our study we found that there is antifungal effect of natural honey against *C.albicans* in low concentration while the effect of synthetic honey and sugar syrup in high concentration . The MIC of natural honey against *C.albicans* ranged between 20 % to 40%(v/v%) Table 1 .Other study found that the MIC of natural honey against *candida albicans* ranged between 40%- 45% (w/v%)(3) .

While Cavanagh Beazley and Ostapowicz (11) found that a 100% (v/v) concentration of honey had a complete fungicidal effect on *C. albicans*

In one study the MIC for honey ranged between 38% and 42% (v/v) (12). These results

closely to our study these mean that honey is a natural product that is used for its antifungal activity (5)

In order to prove that the antifungal of natural honey coming from special material not only from high concentration of sugar we using a synthetic honey and sugar syrup we found that there is a less effective antifungal against *C.albicans* the MIC over 60% (v/v%) . Molan (6) has studied sugar syrups too and found that there is a less effective than honey at inhibiting microbial growth *in vitro*.

Our results proved that the inhibitory effect of honey belong to active material not only to only high concentration of sugar. 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 (13).

Conclusion :

Of this study was to examine the antifungal action of honey samples against *C. albicans*, a common inhabitant in the oral cavity humans.

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