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Antifungal Activity of Four Honeys of Different Types from Algeria Against Pathogenic Yeast: *Candida albicans* and *Rhodotorula sp.*

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Abstract: The traditional medicine still plays an important role in the primary health care in Algeria. Four Algeria honeys of different botanical origin were analyzed to test antifungal effect against *Candida albicans* and *Rhodolorula sp*. Different concentrations (Undiluted, 10, 30, 50 and 70 % wt/vol) of honey were studied *in - vitro* for their antifugal activity using *Candida albicans* and *Rhodolorula sp*. The range of the diameter of zone of the inhibition of various concentrations of tested honeys was (7-23 mm) for Rhodotorula sp., while *Candida albicans* showed clearly resistance towards all concentrations used. While the MICs of tested honey concentrations against *Candida albicans* and *Rhodolorula sp*. were (70.09-93.48) and (5.65-99.70) % vol/vol, respectively. This study demonstrated that, in vitro, these natural products had clearly an antifungal activity against *Rhodotorula sp*.

Key words: Honey · Antifungal · *Candida albicans* · *Rhodtorula Sp.*

INTRODUCTION

The increase in the resistance of antifungal drugs in use has attracted the attention of the scientific community. Candida species are the most common opportunistic fungal pathogens in humans, with *Candida albicans* being the most prevalent pathogen in mucosal and systemic fungal infections [1]. In addition to *C. albicans*, *Rhodolorula sp*. has been implicated as the ethiologic agent of central venous catheter infection and fungemia [2, 3]. In recent years, there has been an increasing search for new antifungal compounds due to the lack of efficacy, side effects and or resistance associated with some of the existing drugs [4, 5].

Recently, the potential antifungal effect of honey have attracted serious attention within the scientific community [6]. Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide [7, 8]. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [9].The *in vitro* antifungal activity of honey was reported by Maria *et al*. [10], Who observed that honey stops the growth of *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans*. Obaseik-Ebor and Afongo [11] compared the antifungal activity of honey distillate with some antimycotic preparations against *Candida albians* and found that all the straint resistant to conventional antimycotic agents are inhibited by the active fraction of honey distillate.

However, only limited data are available on the susceptibility of *Rhodotorula sp*. to antifungal and antiseptic agents [12, 13]. This study aimed to confirm the usage of Algeria honey as antifungal and antiseptic agents and evaluated this inhibitory action at different honey concentration against *Candida albicans* and *Rhodototorula sp.*

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MATERIALS AND METHODS

Honey Samples: During the 2011 flowering seasons, four honey samples were gathered and provided by various bee-keepers from two area different from the Algeria west. These honey samples were aseptically collected in sterile screwed cups and kept in a cool and dry place (at room temperature) overnight before they were finally transported to the laboratory.

Preparation of Honey Solutions: Honey solutions were prepared immediately prior to testing by diluting honey to the required concentrations (undiluted, 10, 30, 50 and 70%, wt/vol). 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 [14].

Yeast Strains and Susceptibility Testing: Yeasts were maintained on Sabouraud dextrose agar (SDA) 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 standard. Dilutions of these suspensions were subcultured on SDA to determine the number of cfu/ml. The adjusted inoculum was 1 x 10^7 - 5 x 10^7 CFU/ml.

Antifungal Assay: Three different methods were used to evaluate the antifungal activity of honey: disc diffusion, well and spectrophotometric [15].

Antifungal activity of honey was evaluated using agar disc diffusion method against test microorganisms. 100 µl of fresh culture suspension of the test microorganisms was spread on the respective media Sabouraud dextrose agar plates. The concentration of cultures was 1 × 10^7 CFU/ml. For screening, sterile 5 mm diameter filter paper discs were impregnated with 10 µl of honey equivalent to 0.1 mg of honey after being placed on the surface of the inoculated media agar plates. The plates were stood at 4°C for 2 h before being incubated under optimum conditions 37°C for 24 h. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. The diameters of the inhibition zones were measured in millimeter, including the diameter of disc. The controls were set up with equivalent quantities of water.

The agar well diffusion method was employed. The honey samples were first inoculated separately on standard nutrient media with no test organisms so as to evaluate their possible contamination. Thereafter, solidified nutrient agar plates were separately flooded with the liquid inoculums of the different test organisms using the spread plate method. The plates were drained and allowed to dry at 37°C for 30 min after which four equidistant wells of 5 mm in diameter were punched using a sterile cork borer at different sites on the plates. 50 ul of the different concentrations (undiluted, 30, 50 and 70% wt/v) of the honey samples were separately placed in the different punched wells with 1 ml sterile syringe. The plates were allowed to stay for 15 min for pre-diffusion to take place followed by an overnight incubation that lasted for 24 h at 37°C. The diameter of inhibition zones, including the diameter of the well, was recorded. Each assay was carried out in triplicate.

Minimum Inhibitory Concentration (MIC) Determination: Concentrations of honey suspensions (undiluted, 10, 30, 50 and 70%) were incorporated into media to test their efficiency against *C. albicans* and *Rhodotorula. sp*. Each plate reaching final volume of 5 ml incluging both honey and media was inoculated and incubated at 37°C for 48 h. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain would not grow. All MIC values were expressed in % (vol/vol).

RESULTS

Table 1 shows the inhibition zone sizes produced by various honeys at different dilutions. The diameters of zone of the inhibition (ZDI) of honey with various concentrations tested for *Rhodotorula sp*. ranged from 7 to 13 and 8 to 23 mm for disc and well diffusion method, respectively. While *Candida albicans* showed resistant towards all honey concentrations used by both methods. The MICs ranges of the tested honey concentrations were (70.09-93.48) and (5.65-99.70) % vol/vol against *Candida albicans* and *Rhodolorula sp*., respectively (Table 2).

DISCUSSION

The conventional treatment of fungal disease is limited and part of the reason is due to the limited spectrum of the currently antifungal drugs and the expensive treatment, particularly due to the need of

Table 1: Antifungal activity of honey at different concentrations against *C. albicans* and *Rhodotorula sp*

		Inhibition zone diameter (mm)							
		Disc				Well			
	Honey dilution	Honey A	Honey B	Honey C	Honey D	Honey A	Honey B	Honey C	Honey D
Candida albicans	Undiluted	ND	ND	ND	ND	ND	ND	ND	ND
	70%	ND	ND	ND	ND	ND	ND	ND	ND
	30%	ND	ND	ND	ND	ND	ND	ND	ND
	50%	ND	ND	ND	ND	ND	ND	ND	ND
	10%	ND	ND	ND	ND	ND	ND	ND	ND
Rhodotorula sp.	Undiluted	09	08	10	08	22	20	23	14
	70%	ND	ND	ND	ND	ND	ND	ND	ND
	30%	09	08	11	09	13	10	11	08
	50%	13	10	10	07	15	14	18	17
	10%	08	08	07	07	12	11	10	11

ND = No inhibition was detected

Table 2: Minimum inhibitory concentration (MIC) of honey at different concentrations against *C. albicans* and *Rhodotorula sp*

	Candida albicans MIC% (vol/vol)				*Rhodotorula sp* MIC % (vol/vol)			
Honey Concentration	Undiluted	50% (v/v)	25% (v/v)	12.5 % (v/v)	Undiluted	50% (v/v)	25% (v/v)	12.5 % (v/v)
Honey A	81.16	70.09	>100	>100	87.30	96.37	25.04	>100
Honey B	91.36	73.94	>100	>100	89.76	94.63	43.56	>100
Honey C	93.48	75.18	>100	>100	56.14	99.70	5.65	>100
Honey D	84.30	79.27	>100	>100	94.12	80.50	4.90	>100

prolonged therapy. In recent years, several studies on the *in vitro* susceptibility of superficial mycoses to antifungal drugs have been done and the results have shown considerable variation [16]. Thus, nowadays many research are focused on the therapeutical properties of natural compounds [1]. Honey is a natural product that is used for its antifungal activity [6]. Several factors may influence the antifungal activity of honey. For example, DeMera and Angert [18] reported that honey from different phytogeographic regions vary in their ability to inhibit the growth of yeasts, suggesting that botanical origin plays an important role in influencing the antifungal activity. In addition, there are a great variety of components, including phenolic acids, flavonoids and other biomolecules, in different honeys. Biological activity of honey is mainly attributed to the phenolic compounds Estevinho *et al*. [19]. In fact, the antimicrobial action of phenolics is well known and it is related to their ability to denature proteins, being generally classified as surfaceactive agents. Xesus and Maria [20] suggest that the honey mechanism for fungal growth inhibition is not related to the osmotic shock derived from the presence of sugar in the culture medium. Moreover, Wahdan [21] stated that high sugar concentration in honey leads to the high osmolarity that produces antimicrobial activity. Additionally, he found no inhibitory activity of the sugar solutions against *Trichophyton mentagrophytes* and *C. Albicans* and added that fungi are generally much more tolerant than bacteria to the high osmotic effect. Diekema *et al*. [22] reported the *in vitro* activities of 8 antifungals against 64 Rhodotorula isolates. Rhodotorula strains were resistant *in vitro* to fluconazole (MIC50, 1128 mg/ mL) and caspofungin (MIC50, 18 mg/mL). In the present study, *Rhodotorula sp* was susceptible to honey since growth inhibition was reached at the minor level. Honey samples inhibited completely the growth of *Rhodotorula sp.*

Our results showed that undiluted honey was able to inhibit the growth of many species of *Rhodotorula sp* but there was no effect on *C. albicans*. Al-Waili [23] found that honey concentration ranging from 30 to 50% inhibits the growth of several pathogenic microorganisms, including *C. Albicans*. Irish *et al*. [6] reported antifungal efficacy of various honeys against clinical isolates of *C. albicans*, *C. glabrata* and *Candida dubliniensis*. Khosravi et *al.* [24] reported that honey has antifungal activity against Candida species such as *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *Candida kefyr*, *C. glabrata* and *C. dubliniensis*. The results of this preliminary study demonstrated that Algeria honey is an effective inhibitor of *Rhodotorula sp.*

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