



Comparing the Antimicrobial Potential of Sahara Honey from Algeria and Manuka Honey against Urogenital Microorganisms

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MA and MAA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors SA and ND revised the manuscript number of times. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2016/27608

Editor(s):

(1) Nawal Kishore Dubey, Centre for advanced studies in Botany, Banaras Hindu University, India.

Original Research Article

Received 10th June 2016
Accepted 23rd July 2016
Published 31st July 2016

ABSTRACT

Aims: Various studies have been conducted to investigate the antimicrobial properties of honey from different parts of the world. To date; no extensive studies of the antimicrobial properties of Sahara honey (SH) on urogenital microorganisms have been conducted. The objectives of this study were to conduct such studies and to compare the antimicrobial activity of SH with Manuka honey (MH).

Place and Duration of Study: This study was conducted in the experimental laboratories at Pharmacognosy and Api-Phytotherapy Research Laboratory, Mostaganem University, Algeria, between April to May 2015.

Methodology: Several unifloral SH and MH were analyzed to determine their total phenolic, color and antimicrobial capacities. The Folin-Ciocalteu assay was used to measure phenol content. Two different assays were performed to evaluate the antimicrobial potential of the honey samples: agar-well and disk diffusion assay. The honey samples were tested without dilution, and at 50 and 25% (w/v) dilution.

Results: The means of the total phenolic contents of SH and MH were 82.8 ± 0.23 and 143.5 ± 0.62 mg/100 g honey as Gallic acid equivalent, respectively. Initial screening with the agar-well and disk

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diffusion assay demonstrated that undiluted honey had greater antimicrobial activity against all isolates tested. The zones of inhibition values of SH and MH against different strains ranged from 15 to 27.5 mm and 16.5–24 mm respectively. In addition, honey showed inhibition zone larger toward entire isolates when mixed. This is the first report on antimicrobial effect of SH against urogenital microorganisms.

Conclusion: This work demonstrates the potential of Sahara honey is a very good trend in the treatment for polymicrobial infections.

Keywords: Antimicrobial activity; Sahara honey; Manuka honey.

1. INTRODUCTION

Bee products have attracted the attention of researchers due to its biological properties, such as antimicrobial, immunomodulatory, anti-inflammatory, antitumor, among others [1-2]. To date, various studies have been conducted to investigate the antimicrobial properties of honey bee from different parts of the world [3-5]. The antimicrobial activity has been reported against a wide variety of fungi. In traditional medicine in Algeria and some other countries, this bee product is used for the treatment applications. The molecules of honeys responsible for their antimicrobial activity are high sugar content, flavonoids, phenolic acid, hydrogen peroxide, enzymes like catalase and peroxidase and finally some products of Maillard reaction [6]. Limited studies have been done on Algerian Sahara honey. In brief, Ahmed et al. [7] studied antimicrobial activities of six local honey varieties against various pathogenic bacteria strains. Manuka honey, which is produced in Australia and New Zealand, is effective against several human pathogens including *C. albicans* and *P. mirabilis*.

Urogenital pathogenic *C. albicans* and *P. mirabilis* are among the frequent agents of urogenital tract infections in humans [8-9]. No study has been undertaken to test the antimicrobial efficacy of Sahara honeys against urogenital pathogenic *C. albicans* and *P. mirabilis*. To our knowledge, this is the first study to reveal the broad spectrum of antimicrobial activities of the local Algerian Sahara and imported honey against *C. albicans* and *P. mirabilis*.

2. MATERIALS AND METHODS

2.1 Honey Samples

Three honey samples were collected in Sahara (South of Algeria) were collected directly from professional beekeepers. All honey collection was conducted between the months of July 2013

and August 2014. Manuka honey with unique manuka factor UMF® 15+ was used as a standard for comparison. The samples were stored at 4°C until analysis in dark conditions.

2.2 Preparation of Honey Solutions

Undiluted and two-fold serial dilutions of honeys (25 and 50 % v/v) were diluted in sterile distilled water bidistilled water in the proportions of Different concentrations A Two-fold serial dilution was prepared using all five pre-filled tubes together with four extra tubes containing honey dilutions of 5, 10, 15 and 20% (w/v). Different concentrations of honey constituting undiluted, 25 and 50 % v/v were made using sterile nutrient broth. All samples were then incubated for 30 minutes 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].

2.3 Colour Intensity: ABS₇₂₀₋₄₅₀

The net absorbance of the SH was determined by the method of Beretta et al. [11]. The SH were diluted to 50% (w/v) with warm (45– 50°C) milli Q water and the solution was filtered through a 0.45 µm filter. There was a complete absence of coarse particles in the honey solutions as all the commercial samples were no crystalline liquid honeys. The absorbance was measured using a spectrophotometer at 450 and 720 nm and the difference in absorbance was expressed as mAU.

2.4 Quantification of Total Phenol Content (TPC)

The total phenolic content of honey was determined by the Folin-Ciocalteu colorimetric assay based on the procedure previously identified by Singleton et al. [12]. Thirty microlitre of honey solution (0.1 g/ml) was mixed with 2.37 ml of milli Q water and 150 µl of 0.2 N Folin–

Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min at ambient temperature. Four hundred and fifty microlitre of sodium carbonate solution (0.2 g/ml) was added to the reaction mixture and further incubated for 2 h at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (0–200 mg/l). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

2.5 Microorganisms and Culture Conditions

Fresh cultures of human pathogens, which included *P. mirabilis* and *C. albicans*, were obtained from private clinic medical analysis, Tiaret Algeria. The clinical strains were obtained from patients with urogenital infections. The isolates were identified by standard microbiological techniques.

The bacterial culture from fresh media were compared with 0.5 McFarland turbidity standards, which is equivalent to approximately 1×10^8 bacterial cell counts per mL and it was maintained throughout the experimentation. For *C. albicans* was cultivated in Sabouraud dextrose agar and were incubated at 37°C for 48 h. Colonies from 48 h cultures were suspended in 5 mL of a sterile-saline solution. The count of yeast was adjusted to yield 1.5×10^8 CFU/mL using the standard McFarland counting method.

2.6 Agar Well Diffusion Assay

In this study two different assays were performed to evaluate the antibacterial potential of the honey samples: Agar-well diffusion (AWD) and Agar disc diffusion (ADD).

Agar-well diffusion assay was used to evaluate the antimicrobial activity of the honey based on Ahmed et al. [13]. Briefly, agar plates (90 mm) were containing 20 mL of Sabouraud dextrose and Nutrient agar plates were inoculated using a swab from a suspension of each organism containing 1×10^8 CFU/ mL. An 8-mm diameter well was cut into the agar and 100 μ L 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. Plates were incubated at 37°C for 48 h. For *P. mirabilis*,

plates were incubated at 37°C for 24 h. After incubation, the diameters of the inhibition zones were measured.

2.7 Agar Disc Diffusion

All honey samples were screened by agar disc diffusion assay as adopted from the work of Ahmed et al. [14]. Briefly, Petri plates were prepared with 20 ml of sterile Sabouraud dextrose and Nutrient agar. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of the honey (undiluted, 25 and 50%) and 100 μ l/ml per disc). The plates were incubated for 48 h at 37°C. For *P. mirabilis*, plates were incubated at 37°C for 24 h. Zones of inhibition were recorded in millimeters and the experiment was repeated twice. The controls were set up with equivalent quantities of water.

2.8 Effects of Honey on Mixed Microbial Culture of Human Isolates

10 μ l specimens of *P. mirabilis* and *C. albicans* were cultured into 10 ml broth containing (Undiluted, 25 and 50%, v/v) honey concentrations. Microbial growth was assessed on solid plate media after 24 and 48 hours' incubation respectively.

3. RESULTS AND DISCUSSION

In this study, we compared the zone inhibition diameters (ZID) values of Sahara honey and other honey determined by well diffusion assay. The colour intensity and TPC values of Sahara honey and other honey also were compared. The results obtained showed that the total phenolic content (mg GAE/100 g honey) determined by the modified Folin-Ciocalteu method varied greatly among the honey types, as is apparent from Fig. 2. The means of the total phenolic contents of SH and MH were 82.8 ± 0.23 and 143.5 ± 0.62 mg/100 g honey as gallic acid equivalent, respectively. The inhibitory effect of the tested honeys at various concentrations is shown in Tables 1, 2 and 3. For the activity of 100% pure honey, the largest zones observed were against *P. mirabilis*. SH1 27.5 mm. The zones of inhibition values for *C. albicans* were SH3 (22 mm) while Manuka honey ranged from 24 mm to 16.5 respectively. When *P. mirabilis* and *C. albicans* cultures combined; the cultures became more susceptible to honey. The zones of inhibition values for *C. albicans* and *P. mirabilis*

were SH 32 to 40 mm while Manuka honey ranged from 32 mm to 38 respectively. The concentration of honeys at 25% had no inhibitory effect on the two microorganisms tested. *C. albicans* and *P. mirabilis* are all important pathogens causing various infections and their antibiotic resistance is a significant problem worldwide. This study evaluated the antimicrobial activity of SH samples obtained from different sources against *C. albicans* and *P. mirabilis*. Against *C. albicans* and *P. mirabilis* several honeys with different parts of the world backgrounds have been reported to possess antimicrobial activity [15-16]. SH is readily available in Algeria, but its quality and floral origin have yet to be determined and standardized. Previous reports showed that Algerian SH possessed high antimicrobial activity against wide range of microorganisms [17,13]. However, the efficacy of honey in inhibiting fungal and bacterial strains could be

attributed not only to its floral sources, concentration but also its physicochemical properties. Different studies have evaluated the antimicrobial proprieties of MH against various bacterial strains [18-20]. Cameroonian honey undiluted and at concentrations of 10%,50% and 75% above were found to be inhibitory to all isolates of bacterial tested. Zones of inhibition of growth around the disc margin of the three microbial organisms, tested ranged from 16.6 to 36.6mm in diameter for the undiluted honey and from 8 to 38 mm in diameter for concentrations of honey at 10%, 50% and 75%. For the activity of 100% pure MH, the largest zones observed were against *E. coli* 35 mm. and from 33 mm in diameter for concentrations of MH at 10%- 75% [21]. This research is the first study to report the efficacy of Algerian Sahara honey on polymicrobial culture collected from human specimens.

Table 1. Determination of the effect of various honey concentrations on *C. albicans*

Methods	Mean zones of inhibition (diameter mm including well (8 mm))					
	Well diffusion assay			Disk diffusion assay		
	Concentrations	Undiluted	50%	25%	Undiluted	50%
SH1	18.5	9	8.5	ND	ND	ND
SH2	15	8	10	ND	ND	ND
SH3	22	15	12	ND	ND	ND
MH	16.5	8	12.5	ND	ND	ND
MC	-	-	-	-	-	-

SH: Sahara honey; MH: Manuka honey; NC Negative control, ND: No detected

Table 2. Determination of the effect of various honey concentrations on *P. mirabilis*

Methods	Mean zones of inhibition (diameter mm including well (8 mm))					
	Well diffusion assay			Disk diffusion assay		
	Concentrations	Undiluted	50%	25%	Undiluted	50%
SH1	27.5	21.5	16	8	17	ND
SH2	23.5	20	8	8.5	13	ND
SH3	27.5	22.5	14.5	8	12.5	ND
MH	24	18.5	9	8.5	ND	ND
MC	-	-	-	-	-	-

SH: Sahara honey; MH: Manuka honey; NC Negative control, ND: No detected

Table 3. Determination of the effect of various honey concentrations on *C. albicans* + *P. mirabilis*

Methods	Mean zones of inhibition (diameter mm including well (8 mm))					
	Well diffusion assay			Disk diffusion assay		
	Concentrations	Undiluted	50%	25%	Undiluted	50%
SH1	40	32	ND	38	ND	ND
SH2	36	34	ND	32	ND	ND
SH3	39	30	ND	35	ND	ND
MH	38	ND	ND	32	ND	ND
MC	-	-	-	-	-	-

SH: Sahara honey; MH: Manuka honey; NC Negative control, ND: No detected

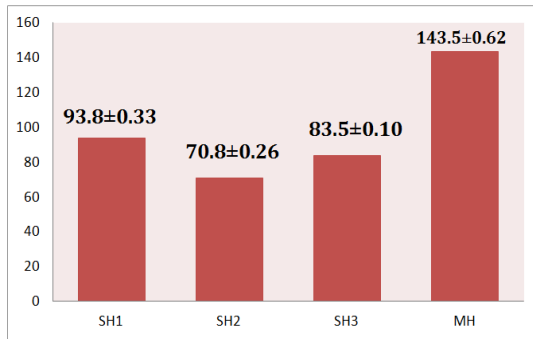


Fig. 1. Total phenolic contents (TPC) in honey samples with Foline Ciocalteu method
 Mean ± S.E.M = Mean values ± Standard error of means of three experiments

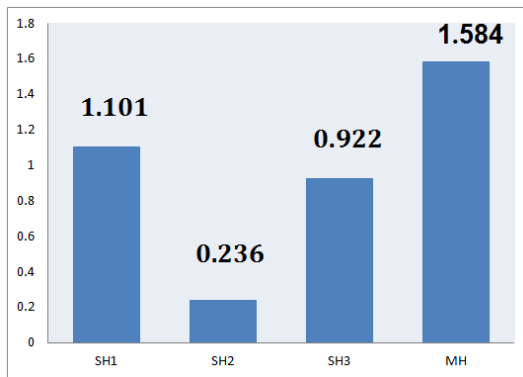


Fig. 2. Color intensity of the Sahara and Manuka honeys
 Mean ± S.E.M = Mean values ± Standard error of means of three experiments

4. CONCLUSION

We concluded that the tested SH possessed favorable antimicrobial against urogenital microorganisms. Further studies should be conducted to determine the active constituents of honey.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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