Research Article

Sensitivity of Clinical Bacterial Isolates to Honey Marketed in Yemen

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ABSTRACT

The clinical bacteria of multi-drug resistant to some antibiotics are considered a common problem in the world wide. Alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey. The first objective of study was to evaluate the antibacterial activity of *Rhamnus Frangula* honey (Sider in Arabic Language) marketed in Yemen against some Clinical Bacterial Isolates (CBIs) and Control Strains of Bacteria (CSB). The second objective to compare between the activity of honey and Augmentin® (Amoxicillin and Clavulanic Acid). The third objective was to determine the sensitivity of CBIs from different regions of patient to different honey concentrations. This study was carried out at during the period of one year from May 2012 to April 2013. The clinical bacteria were isolated (n = 50) from different sources of patients and the antibacterial activity of honey with different concentrations was determined against CBI (*Staphylococcus aureus and Pseudomonas aeruginosa*) and CSB (*Staphylococcus aureus* ATCC25619, *Pseudomonas aeruginosa* ATCC29737) according to disc agar diffusion technique, also the Minimum Inhibitory Concentration (MIC) of honey was determined by using tube agar dilution method. The results indicated that, 30 CBIs (*Staphylococcus aureus*) and 21 CBI (*Pseudomonas aeruginosa*) were sensitive to honey, and also non statistically significant difference between the maximum concentration of the honey and Augmentin® discs ($p < 0.05$) against CBI. This study observed that, there was strong positive correlation between the diameter zone (DZ-mm) and honey concentrations (%). In addition, the DZ (mm) of the honey and Augmentin® for CSB was higher than CBIs and the activity of both antibacterial agents was found to be more effective against *Staphylococcus aureus* than *Pseudomonas aeruginosa*. On the other hand, the MICs of the honey against CBIs and CSB were ranged between 12.5 % and 25%. It could be concluded that, the honey had high antibacterial effect against CSB and CBIs with high concentration.

Keywords: Antibacterial Activity, Augmentin, Honey, Yemen.

INTRODUCTION

Honey is one of alternative treatments for antibiotic-resistant bacteria that has been gaining an increasingly interest. It was used in the medicine of many ancient communities.[1] The ancient Chinese and Sumerians provided the first written prescriptions relating to the medical use of honey dating back to 2000 B.C.[2]

Several different studies have been reported for application of honey as antibacterial, the first study was report by Van Ketel in 1892, followed by Sackett in 1919.[1] The importance honey cannot be over-emphasized as regards their rule in health remedy. Therefore, the application of honey in medicine has been rediscovered and is gaining acceptance as an antibacterial agent for treatment of ulcers, wounds, and other surface infections.[3] The antibacterial activity of honey has been demonstrated against both aerobic and anaerobic bacteria, and it confirmed by numerous scientific studies. Mandal *et al* used the honey against clinical bacterial isolated (CBI) namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enteric.*[4] Also, Abdelmalek *et al* and Sherlock et al described the effect of honey on *Pseudomonas aeruginosa* infection.[5,6] Yasin described activity of honey against *Staphylococcus aureus.*[7] Agbaje *et al* studied conventional use of honey as antibacterial.[8] The antibacterial activity of honey against methicillin-resistant *Staphylococcus aureus* isolated from pus samples was reported by Nagi et al, Poonam *et al* and Sherlock *et al.*[9,10,6] Also in other studies , effect of honey on *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* biofilms was reported by Motior et al and Alandejan *et al.*[11,12] Sunita et al described in vitro effect of some Indian honeys on *Staphylococcus aureus* from wounds.[13] Sriubolmas *et al* estimated antibacterial activity of Honey.[14]

The clinical bacteria of multi-drug resistant to some antibiotics are considered a common problem in the world wide. Alternative antibacterial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey. The first objective was to evaluate the antibacterial activity of Yemeni sider honey against some Clinical Bacterial Isolates (CBIs) (*Staphylococcus aureus and Pseudomonas aeruginosa*) and CSB (*Staphylococcus*

aureus ATCC25619, *Pseudomonas aeruginosa* ATCC29737). Also to determine the Minimum Inhibitory Concentration (MIC) of *Rhamnus Frangula* honey (Sider in Arabic Language). The second objective of this work was to compare the activity of the honey with Augmentin® (Amoxicillin and Clavulanic Acid). The third objective was to determine the sensitivity of CBIs from different regions of patient to honey.

MATERIALS AND METHODS

Collection and provided

Yemeni *Rhamnus Frangula* honey sample (Sider honey - Dawa'ani - Hadhramaut) was gathered and provided by bee-keeper (Alam Al-Asal), Sana'a, Yemen. This honey sample was aseptically collected in sterile screwed cups and kept in a cool and dry place at 25˚C overnight before transport to the laboratory.

Quality control of honey

Quality control (QC) of honey was performed to obtain information about the possible high quality properties physicochemical properties) of the sampled honey. The quality properties was carried according to the European pharmacopeias (EP) namely identification of glucose, fructose and sucrose by Thin Layer Chromatography (TLC), also pH, refractive index, optical rotation, conductivity and 5-Hydroxymethylfurfural.[15]

Preparation of honey concentration for sensitivity test

Honey solution was prepared immediately before testing by diluting honey to serial double dilution concentrations (12.5%, 25%, 50% and 100%, v/v) with distilled water. It was then incubated for 30 minutes at 37°C in a water bath with shaking that allowed aeration of the solution. Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive[16]

Collection of bacterial samples

Fifty CBIs, obtained from different Department of Microbiology in Al-Thawra General Hospital, Atypical Police Hospital and National Center for Public Health Laboratories, Sana'a city, were isolated from different sources of patients namely pus, ear discharge, urine, nasal, semen and throat.

Identification and preparation of bacterial strains

The isolates were identified based on standard microbiological techniques, and sub-cultured in nutrient agar slope at 37°C under aerobic condition for 24 hour and stored in refrigerator at 4°C until used. Active cultures for experiments were prepared by isolating a loop full of cells from each stock culture of tested bacteria and emulsify in 3-4 ml of sterile physiological saline to a turbidity that matches 0.5 McFarland standard (10^6 Colony Forming Unit (CFU)/ml).[17]

Antimicrobial activity

The antimicrobial activities of honey against CBIs and CSB were measured using a disc diffusion method (Kirby Bauer's disc diffusion technique) according to the National Committee for Clinical Laboratory Standards.[18] What man filter paper was used to prepare discs (6 mm diameter). The filter paper discs were sterilized by autoclaving, then the discs were impregnated in different concentration of Yemeni honey. After that, prepared discs were stored at 4°C in the refrigerator until use. To avoid any condensation the discs were kept at room temperature for one hour before use. A loop full of the prepared bacterial suspensions were separately applied to the center of a sterile Mueller-Hinton plate and spread evenly using a sterile dry cotton wool. The discs were placed and the plates were incubated at 37°C for 24hr. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the disc. The positive control (Augmentin® 30µg disc) and negative control (disc impregnated with distilled water) were also included for each experiment.

Minimum Inhibitory Concentration measurement of honey

The MICs of the honey for different bacteria were determined by tube dilution techniques in Müller-Hinton broth. The honey concentrations were in-corporated into Müller-Hinton broth media to test their efficiency against CBIs (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and CSB (*Staphylococcus aureus* ATCC 25619 and *Pseudomonas aeruginosa* ATCC 29737). Each series of dilutions was inoculated with 10^6 (CFU/ml) of the tested bacteria and incubated at 37°C for 24hr before determining the lowest concentration that inhibited the appearance of visible growth. The highest dilution that exhibited no visible growth was recorded as the MIC. The broth without growth from the MIC procedure was streaked onto subculture of each tested honey concentration.[18]

Statistical analysis

Data obtained were analyzed by using the SPSS Version 15 (Social Package of Statistical Science). The results were expressed as Mean ± SD (Standard deviation). Differences in variables were tested by using independent T-test. The interrelationships between parameters were analyzed by Pearson Correlation Coefficient test (R^2). The significant differences were indicated if the probability value ($p < 0.05$).

RESULTS

Quality of Honey

The quality control assessment of honey was done in this study to evaluate the quality of honey namely sider market of Yemen country. Findings of the present study showed that the honey product sampled from Hadhramaut Governorate, Yemen (collected from

Dawa'ani-valley) were found to be of high quality, glucose, fructose and sucrose were identified by TLC, an intense brown zone due to sucrose, an intense greyish – yellow zone due to glucose, a brown zone due to sucrose, also pH, refractive index, conductivity, optical rotation, 5-Hydroxymethylfurfural were summarized in Table 1 were consisted with EP standard.[15]

Table 1: Quality Data of Honey

Physicochemical Properties	Tests	Reference
TLC		
Glucose	Confirm	Intense brown zone
Fructose	Confirm	Greyish – yellow zone
Sucrose	Confirm	Brown zone
Refractive index	1.47	Minimum 1.487
Conductivity	650 µS·cm-1	Maximum 800 µS·cm-1
Optical rotation	+ 0.4 °	Maximum + 0.6 °.
5-Hydroxymethylfurfural	70 ppm	Maximum 80 ppm

Clinical Bacterial Isolates

In this study, antibacterial activity of oil against some clinical and control strains bacteria was evaluated. Fifth samples were collected from different Department of Microbiology in Al-Thawra General Hospital, Atypical Police Hospital and National Center for Central Public Health Laboratories in Sana'a city. The basic information of the collected samples is shown in Table 2 and Figure 1. The bacteria were isolated by different human specimens (urine, pus, ear discharge, nasal, semen and throat). The most bacteria isolates were given in this table namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antibacterial activity of honey

The activity of the maximum concentration of the honey against CBIs (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) was measured by using DZ (mm) and the results were shown in Table 3. However, this parameter reduced gradually with low concentration of honey and raised with high concentration. In addition, the minimum concentration of the honey was 12.5% for *Staphylococcus aureus* and the response was not available for *Pseudomonas aeruginosa* at the same concentration. On the other mean, there was strong positive correlation between the DZ and honey concentrations (Table 3). As regard as, the DZ of honey and Augmentin® in CSB was higher than in CBI. Furthermore, the DZ of antibacterial agent for Gram positive was higher than Gram negative for both CBIs and CSB.

Table 2: Tested CBI and their sources

Bacterial isolated	Urine	Pus	Ear discharge	Nasal	Semen	Sputum	Total
S. aureus	2	19	17	4	3	5	50
P. aeruginosa	5	26	12	0	0	7	50

CBIs: Clinical Bacterial Isolates; *S. aureus*: *Staphylococcus aureus; P. aeruginosa: Pseudomonas aeruginosa*

Table 3: Correlation between DZ (mm) ± SD and honey concentrations (%) by Pearson correlation coefficient

Name of Bacteria	12.5 %	25 %	50 %	100 %	Augmentin® 30µg	R^2
S. aureus	11.0±5.9	16.8±6.7	22.8±4.1	28.0±3.8	27.1±6.0	0.951
P. aeruginosa	0	8.9±4.6	16.3±5.4	21.7±4.0	20.3±3.6	0.922
S. aureus ATCC 25619	15±0	22±0	32±0	38±0	35±0	0.943
P. aeruginosa ATCC 29737	5±0	10±0	20±0	27±0	25±0	0.920

R^2: Pearson's correlation coefficient (≥ 0.995); **DZ**: Diameter Zone

Also, the results showed that the maximum concentration of the honey had high activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in compared to Augmentin® and other honey concentrations (Table 4 and 5) and all results of both tables proved the existence of statistically significant difference ($p < 0.05$) between the honey concentrations and Augmentin® discs except at 100 % was no significant difference ($p > 0.05$). In brief, the results proved that the maximum concentration of the honey had the same antibacterial activity of Augmentin® (Table 4 and 5).

Minimum Inhibitory Concentration of honey

Finally, the results showed that the of MIC of honey was 12.5 % against *Staphylococcus aureus*. In contrast, the MIC of honey was 25 % against *Pseudomonas aeruginosa* (Table 4, 5, 6).

Sensitivity of Clinical Bacterial Isolates from different regions of patient to honey

Fifty *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from urine, pus, ear discharge, semen and throat. Table 6 indicated that the sensitivity of *Staphylococcus aureus* for honey was at 12.5%, followed by 25 %, 10.5%, 100%. On the other hand, the sensitivity of *Pseudomonas aeruginosa* for honey was at 25%, followed by 50 % and 100%. Table 6 showed 54% of *Staphylococcus aureus* and 66% of *Pseudomonas aeruginosa* clinical isolates did not sensitive to any concentration of honey (Resistance). On the other mean,

the sensitivity of Gram negative for honey concentrations was lower than the sensitivity of Gram positive.

Table 4: Comparison of antibacterial activity between honey concentrations and Augmentin® against *S. aureus* according to DZ (mm) ± SD, (n = 50)

Honey (%)	S. aureus	Augmentin® 30µg	*p* value
12.5	11.0±5.9	27.1±6.0	0.000*
25	16.8±6.7		0.000*
50	22.8±4.1		0.002*
100	28.0±3.8		0.4

***:** Statistically Significant

Table 5: Comparison of antibacterial activity between honey concentrations and Augmentin® against *P. aeruginosa* according to DZ (mm) ± SD, (n = 50)

Honey (%)	*S. aureus*	Augmentin® 30µg	*p* value
12.5	0	20.3±3.6	0.000*
25	8.9±4.6		0.000*
50	16.3±5.4		0.002*
100	21.7±4.0		0.4

***:** Statistically Significant

Table 6: Sensitivity of CBIs from different region of patients for different honey concentration

Honey concentration (%)	*S. aureus* n = 50	*P. aeruginosa* n = 50
12.5	4	0
25	26	4
50	16	22
100	0	8
Sensitive (%)	46	34
Resistance (%)	54	66
Total (%)	100	100

DISCUSSION

Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies and ailments of both microbial and non-microbial origins. Among strategies to combat bacteria, the search for new antibacterial drugs including those derived from plants appears to be apriority. Investigations of plants of various genera have provided strong evidence for several compounds with potent antibacterial activity.[19]

There are numerous reports of the antibacterial activity of honey against a wide range of bacterial and fungal species.[20,21] In this study, the effect of sidr honey against CBIs and CSB was found to be more effective on *Staphylococcus aureus* than *Pseudomonas aeruginosa*. This result agreed with study performed by Halwani and Shohayeb which they confirmed the antibacterial effect of honey against the Gram positive bacteria as Staphylococcus *aureus* was more sensitive than Gram negative bacteria as *Pseudomonas aeruginosa*.[22] In contrast, Al-Namma observed that honey has a greater inhibitory effect on Gram negative bacteria that *Pseudomonas aeruginosa* was more susceptible than other Gram negative bacteria.[23] In fact, the effect of honey on Gram negative bacteria was explained by Taormina *et al.,*[24], who attributed it to several properties including osmotic effect, presence of hydrogen peroxide and powerful antioxidants, as also to naturally low pH, which is unsuitable for bacterial growth and to the presence of phenolic acid, lysosome and flavonoids. In addition, however the variation of the activity of different honeys is attributed to the previously mentioned factors, as well as there are other factors such as the kind and amount of honey components, sources of honeys, differences in growth rate of pathogens, nutritional requirements, temperature, inoculum's size and the test method were influence the antibacterial activity.[25]

In the present study, 54 % of *Staphylococcus aureus* and 66 % of *Pseudomonas aeruginosa* clinical isolates did not sensitive, these results may be different genotype of bacteria. The more effect of honey against Gram positive bacteria more than Gram negative bacteria due different of cell wall permeability for both bacteria.[26] The antibacterial activity of honey samples against *Pseudomonas aeruginosa* exhibited that it grows rapidly in low concentrations of honey. These results are comparable with study of Taghizadeh *et al* that showed a direct relation between antibacterial activity and honey concentration.[27] In this study, MIC for CSB were sensitive to low concentrations of Yemeni honey, particularly against *Staphylococcus aureus*. In addition, the DZ of honey for CSB were higher than CBIs. These results indicate that, there are differences in the susceptibility of both bacteria that have the same species. The results of this study were similar to other study performed by Merckoll *et al.* Which this study has confirmed for CBI in compare with CSB.[28]

CONCLUSION

This study concluded that the Yemeni honey is potent antibacterial agents against CBIs and CSB. The highest antibacterial activities of Yemeni honey were at the maximum concentrations but start to reduce with decreasing of concentrations against *Staphylococcus aureus and Pseudomonas aeruginosa*. There was strong positive correlation between the DZ (mm) and concentrations of honey (%). The activity of honey was found to be more effective against *Staphylococcus aureus* than *Pseudomonas aeruginosa* and at the highest concentration has the same effect of Augmentin® discs. This study recommends the following: Yemeni honey could be used for treatment of bacterial infections. Pharmacological standardization and clinical evaluation on the effect of Yemeni honey is an essential before using it as a preventive and curative measure to common diseases related to the tested bacterial species.

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