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Full Length Research Paper

Effectiveness of antibiotics blended with honey on some pathogenic bacteria species

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The antibacterial activity of three local Yemeni honey brands (Sidr, Maraiy and Somor honey) and antibiotics (Gentamicin and Doxycyclinehyclate) were investigated by agar well diffusion method against four standard bacteria isolates: *Escherichia coli* ATCC 10536 (*E. coli*), *Staphylococcus aureus* ATCC 29737 (*S. aureus*), *Pseudomonas aeruginosa* ATCC 25619 (*P. aeruginosa*) and *Salmonella abony* ATCC6017 (*S. abony*), and a comparison of these isolates was made with the effectiveness of antibiotics blended with honey on the growth of all used standard bacteria. All diluted honey brands (25%, 50% and 75%) inhibited growth of 3 standard bacteria (*S. abony*, *S. aureus* then *E. coli*), while *P. aeruginosa* gave moderate growth with effect on its pigment production. The inhibitory effect of Gentamicin on test organisms inhibited the growth of *S. aureus* (17.5 mm), *S. abony* (15 mm) and *P. aeruginosa* (6.5 mm). Mixture of Gentamicin and honey brands showed maximum inhibitory zones (sensitivity) with *S. abony* and *S. aureus* then *P. aeruginosa* as 32, 30 and 16 mm, respectively; whereas Doxycycline hyclate was not effective on the tested organisms except *S. aureus* which showed high sensitivity (30-32 mm) when Doxycycline hyclate was blended with the samples of honey. The obtained results in this study approved the mixture of honey and antibiotics having antibacterial potency able to establish valuable inhibition zones *in vitro* and they were higher in inhibition values than the reference drugs. In conclusion, honey (a natural product) could effectively complement standard antibiotics, especially in cases of pathogenic infections in wounds in general and in burn wounds in particular, with beneficial healing effects.

Key words: Yemeni honey, antibiotics, blended, pathogenic organisms.

INTRODUCTION

Antimicrobial agents are the substances known to have therapeutic effect on microorganisms either as a control, prevention or cure of microbial and non-microbial disease origin. These antimicrobial agents are synthesized by chemotherapeutic substances obtained majorly from microorganisms, plants and some animal products. The failure of these antibiotics has resulted for man to search for more effective sources of natural products. In some cases, they have been found safe and good source of pharmacological effect for man (Omoya and Akharaiyi, 2012).

Honey is a sweet food made from the synthesis of nectar from flowers, plant saps and man waste products. Honey is a mixture of sugars, mainly fructose and glucose, having the highest percentage among other carbohydrates present (Omoya and Akharaiyi, 2012).

Antimicrobial agents with selective toxicity are especially useful as a chemotherapeutic agent in treating infectious diseases and may be a function of specific receptor requirement for drug attachment or it may depend on the inhibition of biochemical events essential to the pathogen but not to the host (Omoya and Akharaiyi, 2012). Other antimicrobial factors subsequently suggested were low protein content, high C/N ratio, acidity, low redox potential, viscosity, and high osmotic pressure (Adeleke and Olaitan, 2006a; Chute et

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Table 1. Local honeys used in the study.

Honeys	Origin of honey	Floral source
Sidr	Dawan-Hadramout	*Ziziphus spinachristi*
Maraiy	Almahweet	Wild types of plants
Somor	Hadramout	*Acacia nilotica* subsp Indica

al., 2010; Ahmadi et al., 2013).

Honey's curative and antimicrobial effects against various diseases and infections have been documented (Adeleke and Olaitan, 2006b). Recently, studies have focused on honey application for various therapeutic purpose such as prevention of infection in wounds or burns (Mullai and Menon, 2007) and it has been ranked higher in antibacterial effect on burn wounds than silver sulphadiazine (Adeleke and Olaitan, 2006b), oral infections, erosion of mucosa (Ahmadi et al., 2013), as an anticarcinogenic agent, anti-leishmanial effects, chest pain, fatigue vertigo, respiratory ailments, measles, period pains, postnatal disorders, male impotence and pharyngitis due to its antibacterial and anti-inflammatory effects (Eteraf-Oskouei and Najafi, 2013). Honey is now available on formularies in many developed countries. Registered products include medical grade honey in tubes, ointments, gels, impregnated onto non-adherent dressings or alginate, and non-sticky flexible honey sheets. All are sterilized by gamma irradiation (Jenkins and Cooper, 2012).

More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes, anaerobes, Gram positive or Gram-negative (Chute et al., 2010; Aurongzeb and Azim, 2011; Ahmadi et al., 2013; AL-Waili et al., 2013), moulds and yeasts with unique properties because of its bacteriostatic and bactericidal effect (Chute et al., 2010; Aurongzeb and Azim, 2011; Ahmadi et al., 2013). In 1892, the antibacterial action of honey which was reported for the first time were two sorts of antibacterial agents or so called inhibines (AL-Waili et al., 2013).

Gentamicin is an antibiotic noted for its activity against Gram-negative bacteria at a concentration of 4.0 µg/ml, while doxycycline is bacteriostatic against a wide variety of organisms, both Gram-positive and Gram-negative. It is used mainly for the treatment of urinary tract, respiratory tract, and gastrointestinal (GI) tract infections (Jantratid et al., 2010).

Available reports do not indicate deliberate comparative studies on honey's antibacterial activity and standard antibiotics, that a combination of honey and antibiotics may be an effective new antimicrobial therapy for chronic infections. Therefore this study highlights the potential of honey or antibiotics and a combinational use of them on selected pathogenic bacteria to develop novel therapies for chronic infections, to both improve efficacy and reduce the risk of antibiotic resistance.

MATERIALS AND METHODS

Bacterial strains

Four standard bacterial strains (*S. aureus* ATCC 29737, *E. coli* ATCC 10536, *P. aeruginosa* ATCC 25619 and *S. abony* ATCC 6017) were used throughout this study. All bacteria isolates were obtained from the stock culture of the Department of Biology, Faculty of Science, Sana'a University, Republic of Yemen.

Honey samples

Three brands of fresh Yemeni honeys were used in antibacterial susceptibility testing including Sidr Dawaney No. 1 (Sidr), Maraiy Mahwetey (Maraiy) and Somor Hadramey No. 1 (Somor) which were taken from different areas of Yemen (Table 1).

Antibiotics

Two types of antibiotics were used in this study and they are as follows:

- Gentamicin sulphate: as a product of Loramycin, Iran, was obtained in ampoule vials (2 ml) from a local pharmacy store. The antibiotic was used in concentrations of 4 µg/ml (aq.).
- Doxycycline hyclate as standard antibiotic was used in concentration of 0.08 ppm (µg/ml).

Biological methods

Preparation of honey samples

This study was carried out with natural, un-treated and unpasteurized honey samples. The samples were originated from blossoms of wild flowers and did not contain artificial preservatives or diluents.

The samples were prepared by diluting each honey with sterile distilled deionized water (v/v) to obtain 25%, 50% and 75%. Moreover, pure natural or undiluted honey was also used as test sample.

Preparation of inoculums

Mueller–Hinton broth was inoculated aseptically with appropriate microorganisms 24 h before testing. This was to ensure that the bacteria fully adapted to the broth and reached the stationary phase of growth. The inoculums bacteria strains were incubated at 37°C during 18-24 h in Mueller–Hinton agar.

After 24 h of incubation, bacterial suspension (inoculums) was diluted with sterile physiological solution to approximately 10^6 CFU/ml by matching with McFarland barium sulfate standard 0.5. The turbidity was visually compared with McFarland 0.5 standard (Becton,

Table 2. Antibacterial activity of three types of honey against standard bacterial isolates (mean ± SD).

Bacterial strain	Honey sample	Honey dilution				75% (SD ±)
		25%	50%	75%	Net	
		Diameter of inhibition zone (mm)*				
E. coli	Sidr	0	11	13.5	0	0.11
	Maraiy	14	22	24	0	0.21
	Somor	14.5	21.5	24	0	0.21
S. aureus	Sidr	0	21.5	26	0	0.34
	Maraiy	14	20.5	26	0	0.34
	Somor	15.5	24	26	0	0.34
P. aeruginosa	Sidr	0	0	18.5	0	0.13
	Maraiy	0	9	12	0	0.12
	Somor	7	10	12.5	0	0.13
S. abony	Sidr	14.5	23.5	28.5	0	0.22
	Maraiy	14	25	28	0	0.21
	Somor	16.5	24	29	0	0.11

0 = No inhibition zone; * mean volume.

Dickinson and Co., MD, USA).

Antibacterial susceptible testing

The agar diffusion method (Halawani and Shohayeb, 2011; Anthimidou and Mossialos, 2013) was used to assess the antibacterial potential of Yemeni honeys, antibiotic and their mixture. 100 µl of the prepared bacterial suspension was spread over plates containing Mueller Hinton Agar (HIMEDIA, India) by sterile cotton swab. With previously sterilized cork borer (6 mm in diameter), wells of equal distance were bored.

It was observed that the 1st group contained 100 µl of pure or diluted honey (25, 50 and 75%), the 2nd group contained 45, 70 and 100 µl of antibiotic (Gentamicin or Doxycycline hyclate) and the 3rd group contained antibiotic blended with honey. All samples were aseptically poured into the wells. The plates were allowed to dry at 4°C for 1 h.

The dishes were then incubated at 37°C for 24 h. Culture growth was monitored over 24 h, and if no growth occurred over 24 h, it is referred to as “no growth” or complete inhibition (Lu et al., 2013). Considerations for the sensitivity and resistance of bacteria were based on the extent of the presence or absence of inhibition zones. The zone of inhibition was taken to be the diameter of the zone visibly showing the absence of growth without the 6 mm hole.

If there was no inhibition, the value of 0 mm was assigned to the test sample. All assays were repeated three times for each honey concentration.

Statistical analysis

All assays were carried out in triplicate. The results were expressed as means ± SD.

RESULTS

The results of antibacterial activity of 3 Yemeni honey samples with four concentrations (25, 50, 75 and 100% v/v) against standard pathogenic bacteria were recorded in Tables 2 to 5. The zone of inhibition was taken to be the diameter of clear zone without the 6 mm hole (diameter of cork borer). The antibacterial activity was classified as: resistant, for diameter lower than 8 mm; sensitive, for diameters from 8 to 14 mm; very sensitive, for diameters from 15 to 19 mm; extremely sensitive, for diameters higher than 20 mm (Moussa et al., 2012). All honey samples showed antibacterial activity on bacterial isolates. Sidr, Maraiy and Somor honey showed maximum sensitivity against *S. abony* ATCC 6017 (28-29 mm), followed by *S. aureus* ATCC 29737 (26 mm) and then *E. coli* ATCC 10536 (13.5-24 mm). Whereas, standard isolate of *P. aeruginosa* (ATCC 25619) gave sensitivity between 12-18.5 mm and it had effect on its pigment production with all honey brands. The largest inhibition zone of standard *P. aeruginosa* in concentration 75% was 18.5 mm with sidr honey, while the minimum inhibition zone showed by Maraiy honey was 12 mm. The samples of raw honey did not show any sensitivity with all bacterial tested, but the bacterial growth was moderate on the medium surface (Table 2).

Table 3. Antibacterial activity of antibiotics against standard bacterial isolates (mean ± SD).

Bacterial strain	Antibiotics	µl			100 µl (SD ±)
		45	70	100	
		Diameter of inhibition zone (mm)*			
E. coli	Gentamicin	0	0	0	0.0
	Doxycycline hyclate	0	0	0	0.0
S. aureus	Gentamicin	11	15.5	17.5	0.01
	Doxycyclinehyclate	0	9	11	0.02
P. aeruginosa	Gentamicin	0	0	6.5	0.03
	Doxycycline hyclate	0	0	0	0.0
S. abony	Gentamicin	8.5	13.25	15	0.10
	Doxycycline hyclate	0	0	0	0.0

0 = No inhibition zone; * mean volume.

Table 4. Antibacterial activity of three types of honey blended with gentamicin against standard bacterial isolates (mean ± SD).

Bacterial strain	Honey sample	Honey dilution**				75% (SD ±)
		25%	50%	75%	Net	
		Diameter of inhibition zone (mm)*				
S. aureus	Sidr	20	28	30	28	0.13
	Maraiy	6	20	22	28.5	0.25
	Somor	15.5	25	27.5	29	0.33
P. aeruginosa	Sidr	7	8	8	11	0.01
	Maraiy	7.5	8	11	13	0.12
	Somor	7	9.5	12	16	0.12
S. abony	Sidr	21	26	32	29.5	0.15
	Maraiy	12.5	21.5	24	26	0.21
	Somor	19	22.5	24	25.5	0.21

0 = No inhibition zone; * mean volume; ** 100 µl of honey dilution blended with 45 µl gentamicin (for *S. aureus* and *S. abony*), or with 100 µl gentamicin for *P. aeruginosa*.

Standard Doxycycline hyclate were not effective on the test bacterial isolates except on *S. aureus* with inhibitory zones ranging from 9-11 mm at 70 and 100 µl, respectively. All the test organisms were susceptible to gentamycin except *E. coli*. *S. aureus* was the most inhibited with zones of inhibition 11, 15.5 and 17.5 mm at 45, 70 and 100 µl respectively, and *S. abony* was also inhibited with zones of inhibition 8.5, 13.25 and 15 mm at 45, 70 and 100 µl respectively. However, *P. aeruginosa* was resistant to organisms (Table 3).

The inhibitory potency of the mixture of 100 µl honey and antibiotics (Gentamicin or Doxycycline hyclate) was measured. It was observed that *S. abony* ATCC 6017 and *S. aureus* ATCC 29737 showed extremely sensitivity with all blended samples and maximum zone of inhibition showed (by 100 µl of sidr honey at concentration 75% blended with 45 µl gentamicin) were 32 and 30 mm respectively. However, the largest inhibition zone of *P. aeruginosa* was 16 mm by 100 µl of Somor honey blended with 100 µl gentamicin (Table 4).

The results in Table 5 showed that the extremely sensitivity for *S. aureus* with all honey brands blended with Doxycycline hyclate and the highest inhibition zones were between 30-32 mm. Generally, gentamicin or doxycycline hyclate activity against organisms is lower than its activity when mixed with raw and diluted honey.

Table 5. Antibacterial activity of three types of honey blended with doxycycline hyclate against standard bacterial isolates (mean ± SD).

Bacterial strain	Honey sample	Honey dilution**				75% (SD ±)
		25%	50%	75%	Net	
		Diameter of inhibition zone (mm)*				
S. aureus	Sidr	13	24	30	32	0.13
	Maraiy	10	23	27	31	0.32
	Somor	22	25	29	30	0.11

0 = No inhibition zone; * mean volume; ** 100 µl of honey dilution blended with 70 µl of doxycycline hyclate.

In the event of therapeutic failure with antibiotics, honey offers a suitable and better alternative in managing infected burns, wounds and other infections.

DISCUSSION

Under the limitations of this study, results demonstrated that natural honey had an antibacterial activity on some standard pathogenic bacterial isolates. This effect is dependent on the concentration of honey used.

Three of the imported Yemini honeys (Sidr, Maraiy and Somor) were evaluated for their antibacterial potential using agar diffusion technique. The antimicrobial activity of honey was reported to be due to osmotic effect, acidity, hydrogen peroxide and phytochemical factors (Aurongzeb and Azim, 2011; Moussa et al., 2012). Mechanisms of antimicrobial activity of honey are different from antibiotics, which destroy the bacteria's cell wall or inhibit intracellular metabolic pathways. The antibacterial activity is related to four properties of honey. Firstly, honey draws moisture out of the environment and thus dehydrates bacteria. The sugar content of honey is also high enough to hinder the growth of microbes, but the sugar content alone is not the sole reason for honey's antibacterial properties. Secondly, the pH of honey is between 3.2 and 4.5, and this acidity is low enough to inhibit the growth of most microorganisms (Salwa and Maher, 2014). Hydrogen peroxide produced by the glucose oxidase is the third and probably the most important antibacterial component, although some authors believe the nonperoxide activity to be more important. Lastly, several phytochemical factors for antibacterial activity have been identified in honey (Eteraf-Oskouei and Najafi, 2013), and the different honeys result in their varying antimicrobial effects (Moussa et al., 2012). Moreover, its possibility might be related to the differences in susceptibility of each species of microorganism to the antibacterial activity of honey used, and also possibly be due to the different floral sources utilized by the bees and the geographical factors like temperature and humidity of the area where the honey was produced (Tumin et al., 2005).

Raw honey did not show obvious inhibition with bacterial species due to viscosity that limits oxygen dissolving in honey, and it has a negligible level of hydrogen peroxide (Molan's, 2012; Anthimidou and Mossialos, 2013). This is because hydrogen peroxide that has been formed in honey disappears as a result of reaction with other components of the honey.

Glucose oxidase is practically inactive in raw honey. It becomes active to form hydrogen peroxide only when the honey is diluted (White et al., 1963; Aurongzeb and Azim, 2011), because enzymes need a sufficiently high level of free water to be active (Alston and Freedman, 2002) by a factor of 2,500-50,000, thus giving "slow-release" of antiseptics at a level which is antibacterial (Aurongzeb and Azim, 2011). Moreover, in undiluted honey, the water present is almost all bound up on the sugar molecules. During dilution of honey by sterilization of double distillation water, the honey produces H_2O_2 which is an antimicrobial agent. The first indication that there was something more involved than osmosis was the discovery by Sackett in 1919 that the antibacterial potency of honey increased rather than decreased by dilution of honey with water, an observation that was hard to explain (Molan's, 2012).

Therefore, the results of this study were in agreement with those of previous studies which reported that the antibacterial activity of honey increased when the honey was diluted (Sherlock et al., 2010; Halawani and Shohayeb, 2011; Mandal and Mandal, 2011; Kuncic et al., 2012). However, there are few studies whose results do not agree with this study's results (Sharma et al., 2012; Ahmadi et al., 2013). An explanation for the difference of results may be due to the methodological difference between the studies and variation in the composition of the honey being used (Lusby et al., 2005; Ahmadi et al., 2013).

Application of natural honey for the inhibition of microorganisms might be a substitute way in some suitable cases for topical application for certain partially systematic infections (Aurongzeb and Azim, 2011). Molan (2000) found contrasting results in favour of honey on a higher antibacterial activity for honey than silver sulphadiazine in the treatment of bacterial infections of burn wounds.

A combination of the antimicrobial properties of clinically approved antibiotics and the antibacterial activity

of honey could lead to a new spectrum of antimicrobials than when used in single form, such that it has the potential to prevent the emergence of resistant bacterial strains, providing broad-spectrum coverage and consequently improving therapeutic efficiency (Mu¨ller et al., 2013). This emphasized that combination of two or more substances with medicinal values could be better if their components will not cause a reaction that could cause health disaster than healing; hence it will be used to remedy multiple actions of some illnesses by certain pathogens in man. In this work, the recommended dose is 75% of honey which can be used in clinical practice.

Conclusion

The obtained results in this study approved honey and antibiotics having antibacterial potency as able to establish valuable inhibition zones *in vitro*. In this work, the recommended dose was 75% which can be used in clinical practice. Therefore, this study will help in preparation of novel antibacterial drugs using natural products blended with antibiotics.

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