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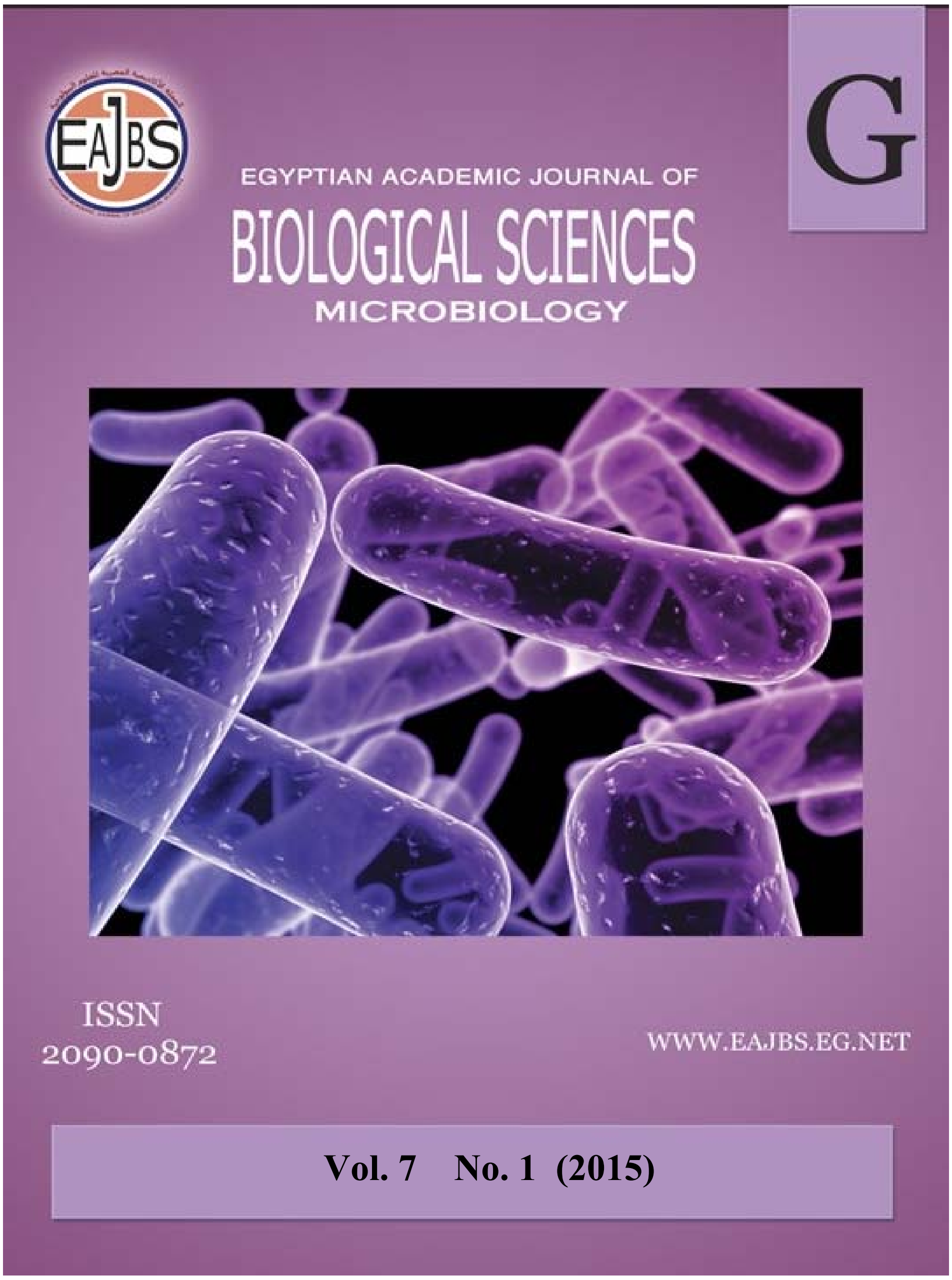

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Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.7 (1)pp. 101-109(2015)*

Egypt. Acad. J. Biolog. Sci., 7(1): 101 – 109 (2015)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872

www.eajbs.eg.net

Synergistic Effects of Honeys and Commonly Used Antibiotics on Gram Positive Bacteria

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ARTICLE INFO
Article History
Received: 1/11/2015
Accepted: 10/12/2015

Keywords:
Honey ,
Antibiotics ,
Gram Positive Bacteria,
Synergistic Effects.
Antibacterial Activity

ABSTRACT

This study aimed to investigate the growth inhibitory effect of sidr and sommor honeys and to evaluate the synergistic effects of various honey concentrations and five antibiotics on gram-positive bacteria in vitro using agar well diffusion and disk diffusion techniques. This is the first time for studying the antibacterial activity and synergistic effect of sommor honey against bacteria. The results indicated that sidr and sommor honeys had antibacterial activity against the clinical isolates and reference strains of *S. aureus* and *Strept. pyogenes*. The reference strains were more susceptible to sidr honey than sommor honey while the clinical isolates were more susceptible to sommor honey than sidr honey. Increasing honey concentrations either alone or in combination with antibiotics was significantly increased ($P<0.05$) the growth inhibition of the tested bacteria. The synergistic effects of honeys with antibiotics were significantly ($P<0.05$) different among the tested gram-positive bacteria. The highest synergistic effect was observed against *Strept. pyogenes* clinical isolate when sidr and sommor honeys combined with ofloxacin, pipracillin, amoxicillin + clavulanic acid and sulphamethoxazole + trimethoprim. It can be concluded that sidr and sommor honeys improved the antibiotic activity and presented a new avenue for treatment of gram-positive bacterial infections.

INTRODUCTION

Infectious diseases continue to take a toll on human health and life expectancy. Treatment of these infections is becoming increasingly difficult due to antibiotic resistance to currently available drugs (Saleem *et al.*, 2010). The emergence in recent years of numerous resistant strains of pathogenic bacteria to a range of formerly efficient antibiotics constitutes a serious threat to public health (Raymond *et al.*, 2011). Honey originally used by the ancient Egyptians and Greeks, honey is a viscous, saturated sugar solution now widely used in wound care (Simon *et al.*, 2009). Bees and honey are mentioned in the holy Qur'an and there is even one Chapter in the Qur'an named "Surah an-Nahl" meaning bees. Allah Said (what means): "And the Lord inspired the bee, saying: Take your habitations in the mountains and in the trees and in what they erect. Then, eat of all fruits and follow the ways of your Lord made easy (for you)'. There comes forth from their bellies a drink of varying colors wherein is healing for men.

Verily in this is indeed a sign for people who think." (Holy Qur'an 16:68-69). The mechanisms of action of honey are still not fully understood; however, the antimicrobial activity of most honeys is linked to the production of hydrogen peroxide by the enzyme glucose oxidase which combined with high acidity and exerts an antimicrobial effect (French *et al.*, 2005). Honey is an ancient wound treatment that was re-introduced into modern medical practice and possesses therapeutic potential and antimicrobial activity (Andargarchew *et al.*, 2004). There are many reports of bactericidal and bacteriostatic activity of honey against bacteria, which have developed resistance to many antibiotics (Patton *et al.*, 2006). Sidr honey is made from bees who feed only on the nectar of the sidr tree, which is native in the South Saudi Arabia and Yemen regions. Sidr honey has wide medicinal applications and uses which include: liver diseases, stomach ulcers, respiratory infections, malnutrition diseases, digestive problems, constipation, eye diseases, infected wounds and burns. Sidr honey has strong antioxidant and antibacterial properties (Alandejani *et al.*, 2009). By searching the internet, no published papers were recorded for sommor honey, and just short data was reported at the web sites of honey markets. Sommor honey is made from bees who feed on sommor tree (Acacia) in the Hadramout region, Yemen. A large number of honeys are available in the Saudi market and some of them are traditionally used as remedy for several ailments. To date, the antibacterial efficiency of local Saudi honeys has not been thoroughly evaluated (Eman and Mohamed, 2011). To combat antibiotic resistance, combination antibiotic treatment is widely practiced in the clinic. Such treatment can result in synergism to provide increased efficacy and a reduction in amount of each antibiotic used, which can reduce the risk of possible side effects and treatment costs (Lee *et al.*, 2007; Wagner and Merzenich, 2009; Leibovici *et al.*, 2010). A combination of the antimicrobial properties of clinically approved antibiotics and the antibacterial activity of honey could lead to a new spectrum of antimicrobials that have the potential to prevent the emergence of resistant bacterial strains, providing broad-spectrum coverage and consequently improving therapeutic efficiency (Patrick *et al.*., 2013). Moreover, combination use of antibiotics with different modes of action reduce the risk of antibiotic resistance arising during therapy (Raha, 2006). In medical practice, honey might be delivered in combination with traditional antibiotics, with both being administered topically or with honey being administered topically and the traditional antibiotic(s) being administered systemically. Therefore, it is useful to understand the interaction between honey and conventional antibiotics (Michelle and Robin, 2014). Our goals were to assay the growth inhibitory effect of sidr and sommor honeys and to study the synergistic effects of various honey concentrations and antibiotics on gram-positive bacteria in vitro.

MATERIALS AND METHODS

Preparation of honey concentrations, Confirmation of isolates identification, antibacterial assays, antimicrobial susceptibility pattern and evaluation of the synergistic effect of various honey concentrations with antibiotics on gram positive bacteria were conducted at the Microbiology laboratory, Department of Applied Medical Sciences, Community College, Najran University during February to August 2015.

Honey samples and preparation of concentrations

Sidr and sommor honeys were purchased from honey markets at Najran city, Saudi Arabia. Najran is a region of Saudi Arabia, located in the south of the country along the border with Yemen. It has an area of 119,000 km^2. Its capital is Najran. Various concentrations of each honey constituting, 10, 20, 40, 60 and 80% (v/v) were prepared using sterile distilled water.

This was done by dissolving the respective volumes: 1, 2, 4, 6, 8 mL of each honey sample into corresponding volumes of sterile distilled water to give finally a 10 mL preparation.

Bacterial isolates and culture media

Four-gram positive bacteria were used in this study. Two clinical pathogenic bacterial isolates, *S. aureus* and *Strept. Pyogenes* were obtained from the Microbiology laboratory, King Khalid Hospital (K.K.H), Najran region, Saudi Arabia. The organisms were identified by an automated system (Micro Scan Walkaway, Siemens) and the results were confirmed (Koneman *et al.*, 1992). Two reference strains, *S. aureus* ATCC14775 and *Strept. pyogenes* ATCC19615 were obtained from Microbiologics®. The isolates were subcultured on appropriate agar plates 24 hrs. before antibacterial tests. Brain Heart Infusion broth (Oxoid, England), Mueller Hinton agar (Oxoid, England) and Nutrient agar (Oxoid, England) were used in this investigation. Media were prepared according to manufacture recommendations.

Antibacterial activity of honey samples

The growth inhibitory effect of sidr and sommor honeys was determined by the agar well diffusion technique according to NCCLS (1993). Clinical and reference bacterial cultures were grown in Brain Heart Infusion broth at 37 °C for 4 hrs. Each isolate, at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) was inoculated onto the surface of Mueller Hinton agar plates using swabs. The plates were allowed to dry at room temperature; the medium was punched with six millimeters diameter wells. Different concentrations (10, 20, 40, 60 and 80%) of sidr and sommor honeys were checked for growth inhibitory effect by introducing 50 µl into the well and allowed to diffuse at room temperature for 20 minutes. The plates were incubated aerobically at 37° C for 24 hrs. and the inhibition zone diameter (IZD) was measured (mm) using a ruler. Tests were done in triplicate and the growth inhibitory effect of different honey samples and concentrations were recorded. Amikacin (Oxoid) antibiotic disc (30 µg) was used as a positive control.

Antibiotic susceptibility testing

Antibacterial susceptibility pattern was performed using disk diffusion technique (Bauer *et al.*., 1966). Five antibiotics were used in this study (Table 1). Each bacterial isolate, at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) was inoculated by streaking the swab over surface of Mueller Hinton agar plates. The plates were allowed to dry for 5 minutes at room temperature. Then, all antibiotic disks were placed on the surface of the plates and pressed gently to ensure complete contact with agar medium. After 15 minutes of placing disks, the plates were incubated for 24 hrs. at 37 °C. The assessment of bacterial susceptibility was based on measurement of inhibition zone diameter (mm) formed around the antibiotic disk. The experiment was repeated in triplicates for each microorganism.

Table 1: List of antibiotics

Antibiotic	Symbol	Potency(µg)	Company
Ofloxacin	OFX	5	Oxoid
Imipenem	IPM	10	Oxoid
Pipracillin	PRL	100	Oxoid
Amoxicillin + Clavulanic acid (2:1)	AMC	30	Oxoid
Sulphamethoxazole (23.75 µg)+ Trimethoprim(1.25 µg)	SXT	25	Oxoid

Evaluation the synergistic effect of various honey concentrations and antibiotics

Clinical isolates and reference strains at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) were inoculated onto the surface of Mueller Hinton agar plates using swabs. Thereafter, the antibiotic disks of 6 mm in diameter were placed on the surface of each inoculated plate then saturated with 10 µl of various honey concentrations (40, 60 and 80%). The plates were incubated for 24 hrs. at 37°C. Inhibition zone diameters were recorded and compared with that of the antibiotic alone (Betoni *et al.*, 2006).

Statistical analysis

Data were analyzed statistically and were presented as means ± S.E. (Standard Error) using an analysis of variance (ANOVA) according to Fisher's PLSD test. Differences were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Medicinal honeys are registered for topical application in several countries and are formulated in tubes into ointments and gels, and made into wound dressings (Kwakman *et al.*, 2011).

Antibacterial activity of sidr and sommor honeys

The growth inhibitory effect of different sidr and sommor honey concentrations was presented in Tables (2-3). All the tested gram-positive bacteria were susceptible to sidr and sommor honeys at concentrations 80-40%. Basualdo *et al.,* (2007) reported that the bactericidal effect of honey is to be dependent on concentration of honey used and the nature of the bacteria. Khan *et al.,* (2007) mentioned that the antibacterial property of honey is also derived from the osmotic effect of its high sugar content and low moisture content, along with its acidic properties of gluconic acid and the antiseptic properties of its H_2O_2.Various sidr honey concentrations (80-10%) inhibited the growth of *Strept. pyogenes* clinical isolate despite that different sommor honey concentrations (80-10%) exhibited antimicrobial effects against *S. aureus* and *Strept. pyogenes* clinical isolates.

Table 2: Growth inhibitory effect of various sidr honey concentrations and amikacin on gram-positive bacteria (all values in mm).

Bacteria	Sidr honey concentrations					Amikacin
	80%	60%	40%	20%	10%	
S. aureus ATCC 14775	15.33 ± 0.33 ^{a}	13.33 ± 0.33 ^{b}	11.00 ± 0.00 ^{c}	00.00 ± 0.00	00.00 ± 0.00	21.00 ± 0.00
Strept. pyogenes ATCC 19615	16.00 ± 0.58 ^{d}	14.33 ± 0.33 ^{e}	12.33 ± 0.33 ^{f}	00.00 ± 0.00	00.00 ± 0.00	21.33 ± 0.33
S. aureus	16.67 ± 0.33 ^{d}	14.67 ± 0.33 ^{e}	13.00 ± 0.00 ^{b}	00.00 ± 0.00	00.00 ± 0.00	22.67 ± 0.33
Strept. pyogenes	18.33 ± 0.33 ^{g}	16.00 ± 0.00 ^{d}	13.33 ± 0.33^{b}	12.00 ± 0.00 ^{d}	10.33 ± 0.33 ^{c}	23.67 ± 0.33

$^{a-g}$ Values with different superscripts in the same raw and column differ at $p< 0.05$.

Table 3: Growth inhibitory effect of various sommor honey concentrations and amikacin on gram-positive bacteria (all values in mm).

Bacteria	Sommor honey concentrations					Amikacin
	80%	60%	40%	20%	10%	
S. aureus ATCC 14775	12.67 ± 0.33 ^{a}	10.33 ± 0.33 ^{b}	8.67 ± 0.33 ^{c}	00.00 ± 0.00	00.00 ± 0.00	21.00 ± 0.00
Strept. pyogenes ATCC 19615	11.33 ± 0.33 ^{d}	11.00 ± 0.58 ^{d}	8.67 ± 0.33 ^{c}	00.00 ± 0.00	00.00 ± 0.00	21.33 ± 0.33
S. aureus	18.00 ± 0.00 ^{e}	16.00 ± 0.00 ^{f}	14.33 ± 0.33 ^{g}	12.67 ± 0.33 ^{a}	11.00 ± 0.00 ^{d}	22.67 ± 0.33
Strept. pyogenes	35.33 ± 0.33 ^{h}	31.00 ± 0.58 ^{i}	18.00 ± 0.33 ^{g}	15.67 ± 0.33 ^{f}	13.33 ± 0.33 ^{c}	23.67 ± 0.33

$^{a-i}$ Values with different superscripts in the same raw and column differ at $p< 0.05$.

Our findings agree with other observations (Wilkinson and Cavanagh, 2005; Mohammad *et al.*, 2008; Henriques *et al.*, 2010; Maddocks *et al.*, 2012). The reference strains were more susceptible to sidr honey than sommor honey. On the other hand, the clinical isolates more susceptible to sommor honey than sidr honey. Jenkins and Chapagain, (2014) found that the clinical isolate was more sensitive to manuka honey than the reference strain. Taormina *et al.*, (2001) reported that physical properties along with geographical distribution and different floral sources may play important role in the antimicrobial activity of honey. Increasing honey concentrations significantly increased the growth inhibition of the tested bacteria. This result agrees with the result previously recorded by (Alqurashi *et al.*, 2013). The clinical and reference strains were susceptible to amikacin antibiotic with IZDs ranged from 21.00±0.00 - 23.67 ± 0.33 mm.

Antibiotic susceptibility and synergistic effect of various honey concentrations and antibiotics against *S. aureus*ATCC14775 reference strain

All the tested antibiotics inhibited the growth of *S. aureus* ATCC14775 with IZDs ranged from 20.33± 0.33-52.00±0.00 mm (Table 4). It was observed that various sidr and sommor honey concentrations (80-40%) exerted synergistic effects with pipracillin, amoxicillin + clavulanic acid and sulphamethoxazole + trimethoprim. The present findings revealed that sidr and sommor samples at ((80-40%) concentrations did not show synergistic effects with ofloxacin and imipenem on *S. aureus* ATCC14775. Our results agree with other observations (Ali *et al.*, 2005; Patrick *et al.*, 2013).

Table 4: Synergistic effect of sidr and sommor honeys (80 - 40%) with antibiotics on *S. aureus* ATCC14775 (all values in mm).

Antibiotics+ Honeys	Honey concentrations			Antibiotic alone
	80%	60%	40%	
OFX + Sidr	29.33 ± 0.33	29.33± 0.33	29.00 ± 0.00	29.33 ± 0.33
OFX + Sommor	29.33± 0.33 [a]	28.33± 0.33 [b]	28.33 ± 0.33 [b]	29.33 ± 0.33 [a]
IPM + Sidr	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00
IPM + Sommor	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00
PRL + Sidr	26.67 ± 0.33 [c]	25.33 ± 0.33 [d]	25.00 ± 0.00 [d]	20.33± 0.33 [e]
PRL + Sommor	24.00 ± 0.00 [f]	22.33 ± 0.33 [e]	22.33 ± 0.33 [e]	20.33± 0.33 [e]
AMC + Sidr	33.67± 0.33 [g]	32.00 ± 0.00 [h]	30.67± 0.33 [h]	26.67 ± 0.33 [c]
AMC + Sommor	31.00 ± 0.00 [h]	29.33 ± 0.33 [a]	29.00 ± 0.58 [a]	26.67 ± 0.33 [c]
SXT + Sidr	31.67 ± 0.33 [h]	30.00 ± 0.00 [a]	29.00 ± 0.58 [a]	25.33 ± 0.33 [d]
SXT + Sommor	28.00 ± 0.00 [b]	28.00 ± 0.00 [b]	27.67 ± 0.58 [b]	25.33 ± 0.33 [d]

[a-h] Values with different superscripts in the same raw and column differ at $p< 0.05$.

Antibiotic susceptibility and synergistic effect of various honey concentrations and antibiotics against *Strept. pyogenes* ATCC19615 reference strain

The susceptibility of *Strept. pyogenes* ATCC19615 reference strain to Antibiotics and synergistic effect of sidr and sommor honey samples with antibiotics were presented in Table 5. The results showed that *Strept. pyogenes* ATCC19615 reference strain was sensitive to the tested antibiotics. Various sidr and sommor honey concentrations (80-40%) had synergistic effect with pipracillin and sulphamethoxazole + trimethoprim on *Strept. pyogenes* ATCC19615 reference strain.

Table 5: Synergistic effect of sidr and sommor honeys (80 - 40%) with antibiotics on *Strept. pyogenes* ATCC19615 (all values in mm).

Antibiotics+ Honeys	Honey concentrations			Antibiotic alone
	80%	60%	40%	
OFX + Sidr	32.00 ± 0.00 [a]	30.67 ± 0.33 [a]	30.33 ± 0.33 [a]	31.33 ± 0.33 [a]
OFX + Sommor	36.67 ± 0.33 [b]	34.33 ± 0.33 [c]	33.67 ± 0.33 [c]	31.33 ± 0.33 [a]
IPM + Sidr	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00
IPM + Sommor	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00
PRL + Sidr	48.67 ± 0.33 [d]	46.33 ± 0.33 [e]	45.00 ± 0.00 [f]	40.33 ± 0.33 [g]
PRL + Sommor	49.00 ± 0.00 [d]	46.67 ± 0.33 [e]	44.00 ± 0.00 [f]	40.33 ± 0.33 [g]
AMC + Sidr	46.33 ± 0.33 [e]	46.33 ± 0.00 [e]	46.00 ± 0.00 [e]	46.00 ± 0.00 [e]
AMC + Sommor	45.33 ± 0.33 [f]	45.00 ± 0.00 [f]	45.67 ± 0.00 [f]	46.00 ± 0.00 [e]
SXT + Sidr	38.67 ± 0.33 [g]	36.00± 0.00 [b]	34.00 ± 0.58 [c]	31.67 ± 0.33 [a]
SXT + Sommor	36.33 ± 0.33 [b]	34.33 ± 0.33 [c]	34.67 ± 0.67 [c]	31.67 ± 0.33 [a]

[a-g] Values with different superscripts in the same raw and column differ at $p< 0.05$.

No synergism was observed for combination of honey samples with imipenem and amoxicillin + clavulanic acid. Sommor honey had synergistic effect on *Strept. pyogenes* ATCC19615 reference strain when combined with ofloxacin. In contrast, no synergistic effect resulting from combination of sidr honey with ofloxacin. Jenkins and Chapagain, (2014) found that Combining each of the antibiotics with manuka honey resulted in either synergistic or additive activity for a reference strain of *Strept. pyogenes* (NCTC 10085) reference strain and a clinical isolate of *Strept. pyogenes* (74721).

Antibiotic susceptibility and synergistic effect of various honey concentrations and antibiotics against *S. aureus* clinical isolate

The results of the growth inhibitory effect of antibiotics and their synergism with different sidr and sommor honey concentrations on *S. aureus* clinical isolate were presented in Table 6. The present findings revealed that *S. aureus* clinical isolate was sensitive to the five tested antibiotics. Synergistic action was observed when different sidr and sommor honey concentrations (80-40%) were combined with ofloxacin, imipenem and sulphamethoxazole + trimethoprim on *S. aureus* clinical isolate.

Table 6: Synergistic effect of sidr and sommor honeys (80 - 40%) with antibiotics on *S. aureus* (all values in mm).

Antibiotics+ Honeys	Honey concentrations			Antibiotic alone
	80%	60%	40%	
OFX + Sidr	35.67 ± 0.33^{a}	33.67 ± 0.33^{b}	32.00 ± 0.00^{c}	28.67 ± 0.33^{d}
OFX + Sommor	36.33 ± 0.33^{a}	34.33 ± 0.33^{b}	32.00 ± 0.58^{c}	28.67 ± 0.33^{d}
IPM + Sidr	42.00 ± 0.00^{e}	39.33 ± 0.33^{f}	39.67 ± 0.33^{f}	35.67 ± 0.33^{a}
IPM + Sommor	41.33 ± 0.33^{g}	39.33 ± 0.33^{f}	38.67 ± 0.33^{f}	35.67 ± 0.33^{a}
PRL + Sidr	28.33 ± 0.33	27.67 ± 0.33	27.67 ± 0.33	28.00 ± 0.00
PRL + Sommor	28.00 ± 0.00	28.00 ± 0.58	27.67 ± 0.33	28.00 ± 0.00
AMC + Sidr	32.00 ± 0.00^{c}	31.33 ± 0.33^{c}	31.33 ± 0.33^{c}	30.67 ± 0.33^{h}
AMC + Sommor	31.33 ± 0.33^{h}	30.67 ± 0.67^{h}	30.67 ± 0.33^{h}	30.67 ± 0.33^{h}
SXT + Sidr	40.00 ± 0.58^{f}	37.67 ± 0.33^{i}	37.00 ± 0.00^{j}	32.67 ± 0.67^{c}
SXT + Sommor	39.67 ± 0.33^{f}	37.33 ± 0.33^{i}	36.67 ± 0.33^{j}	32.67 ± 0.67^{c}

[a-j]Values with different superscripts in the same raw and column differ at $p < 0.05$.

Combination of various honey samples with pipracillin and amoxicillin + clavulanic acid did not show any synergism on *S. aureus* clinical isolate. Synergism between honey and antibiotics was documented in previous studies (Patrick *et al.*, 2013; Michelle and Robin, 2014).

Antibiotic susceptibility and synergistic effect of various honey concentrations and antibiotics against *Strept. pyogenes* clinical isolate

Synergistic effect of different sidr and sommor honey concentrations (80 – 40%) with antibiotics and sensitivity of *Strept. pyogenes* clinical isolate to antibiotics alone were summarized in Table 7. The present study revealed that the synergism was showed on *Strept. pyogenes* clinical isolate when various honey concentrations were combined with ofloxacin, pipracillin, amoxicillin + clavulanic acid and sulphamethoxazole + trimethoprim.

It was observed that, no synergism was obtained against *Strept. pyogenes* clinical isolate when honey samples were combined with imipenem. Our findings were supported by the results previously reported by (Jenkins and Cooper, 2012; Jenkins and Chapagain, 2014). Our results further show that there was an increase of IZD for the tested microorganisms with the increase of honey concentration either alone or in combination with antibiotics.

Table 7: Synergistic effect of sidr and sommor honeys (80 - 40%) with antibiotics on *Strept. Pyogenes* (all values in mm).

Antibiotics+ Honeys	Honey concentrations			Antibiotic alone
	80%	60%	40%	
OFX + Sidr	37.33 ± 0.33 [a]	36.33 ± 0.33 [b]	34.00 ± 0.00 [c]	28.00 ± 0.00 [d]
OFX + Sommor	36.00 ± 0.00 [b]	34.33 ± 0.33 [c]	32.33 ± 0.33 [e]	28.00 ± 0.00 [d]
IPM + Sidr	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00
IPM + Sommor	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00	52.00 ± 0.00
PRL + Sidr	52.33 ± 0.33 [f]	50.33 ± 0.33 [g]	47.67 ± 0.33 [h]	42.00 ± 0.00 [i]
PRL + Sommor	50.67 ± 0.33 [j]	46.00 ± 0.58 [k]	45.00 ± 0.00 [l]	42.00 ± 0.00 [i]
AMC + Sidr	56.00 ± 0.00 [m]	55.00 ± 0.00 [n]	51.67 ± 0.33 [f]	43.67 ± 0.33 [o]
AMC + Sommor	55.67 ± 0.33 [g]	54.00 ± 0.00 [f]	49.33 ± 0.33 [e]	43.67 ± 0.33 [o]
SXT + Sidr	36.33 ± 0.33 [b]	34.33 ± 0.33 [c]	32.00 ± 0.33 [e]	29.33 ± 0.33 [p]
SXT + Sommor	36.67 ± 0.89 [b]	35.00 ± 0.58 [c]	32.00 ± 0.00 [e]	29.33 ± 0.33 [p]

[a-p] Values with different superscripts in the same raw and column differ at $p < 0.05$.

CONCLUSION

The present study concluded that sidr and sommor honeys at concentrations (80-40%) had antibacterial activity against clinical and reference strains of *S. aureus* and *Strept. pyogenes*. The clinical isolates were more sensitive to sidr and sommor honeys than references strains. Both honey samples in various concentrations (80-10%) inhibited the growth of *Strept. pyogenes* clinical isolate than the other tested bacteria. The growth inhibition of the tested bacteria was significantly increased ($P<0.05$) by increasing honey concentrations either alone or in combination with antibiotics. The synergistic effects of sidr and sommor honeys combined with antibiotics were differed among the investigated organisms. The highest synergistic effect was observed against *Strept. pyogenes* clinical isolate when sidr and sommor honeys combined with ofloxacin, pipracillin, amoxicillin + clavulanic acid and sulphamethoxazole + trimethoprim. Sidr and sommor honeys were effective antibacterial agents and can be used in combination with antibiotics for treatment of gram positive bacterial infections especially *S. aureus* and *Strept. pyogenes*.

Competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All authors participated in designing this study. Elsayed Masoud collected honey samples, drafted and revised the manuscript. Elsayed Masoud and Mohammadeen Alamin performed the experiments. Mohammadeen Alamin conducted the statistical analysis. Abdulrahman Alqurashi collected the clinical isolates. The authors shared in the interpretation of study.

Funding and policy

This study was sponsored and funded by Deanship of Scientific Research, Najran University, Saudi Arabia (Grant Code: NU/ESCI/14/014).

ACKNOWLEDGMENTS

The authors thank Najran University, Saudi Arabia for its financial support of this study. Special thank for Prof. Adil Al – Garrai for advice in statistical analysis.

REFERENCES

Alandejani, T., Marsan, J., Ferris, W., Slinger, R. and Chan, F. (2009). Effectiveness of honey on Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Otolaryngol Head Neck Surg, 141(1):114-8

Ali, A. Al-Jabri., Sumaya, A. Al-Hosni., Basil, C. Nzeako., Zahra, H. Al-Mahrooqi. and Herbert Nsanze. (2005). Antibacterial activity of Omani honey alone and in combination with gentamicin. Saudi Med J, 26(5):767-771

Alqurashi, A. M., Masoud, E. A. and Alamin, M. A. (2013). Antibacterial activity of Saudi honey against Gram-negative bacteria. Journal of Microbiology and Antimicrobials, 5(1):1-5 doi:10.5897/JMA2012.0235

Andargarchew, M., Belay, T. and Fetene, D. (2004). In vitro assessment of the antimicrobial potential of honey on common human pathogens. Ethiop J Health Dev, 18:107 - 11

Basualdo, C., Sgroy, V., Finola, M.S., andJuam, M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microbiol, 124:375-381

Bauer, A.W., Kirby, M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standard single disk method. American J. Clinical Pathology, 45:493-496

Betoni, J.E., Mantovani, R.P., Barbosa, L.N., Di Stasi, L.C.,and Fernandes, J.A. (2006). Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Memorias Institute Oswaldo Cruz, 101:387-90.

Eman, Halawani. and Mohamed, Shohayeb. (2011). Survey of the antibacterial activity of Saudi and some international honeys. J. Microbiolo. Antimicrobials, 3(4): 94-101

French, V. M., Cooper, R. A. and Molan, P. C. (2005). The antibacterial activity of honey against coagulase-negative staphylococci. J Antimicrob Chemother, 56: 228–231

Henriques, A. F., Jenkins, R. E., Burton, N. F. and Cooper, R. A. (2010). The intracellular effects of manuka honey on Staphylococcus aureus. Eur J Clin Microbiol Infect Dis, 29(1): 45-50. doi: 10.1007/s10096-009-0817-2.

Holy Qur'an 16: 68-69

Jenkins, R. and Chapagain, S. (2014). Effect of antibiotics in combination with manuka honey on Streptococcus pyogenes. Poster Presentation Australian Society for Micro-biology Annual Scientific Meeting 2014

Jenkins, R.E.,and Cooper, R.A. (2012). Improving antibiotic activity against wound pathogens with manuka honey in In Vitro. PLOS one. doi: 10.1371/journal.pone.0045600

Khan, F.R., Abadin, U.I. and Rauf, N. Honey. (2007). Nutritional and medical Value. Medscape Today. [Online] Available from: http://www.medscape.com/viewartide/565913

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C. and Winn, W.C. (1992). Packaged in Kit Identification System. Color Atlas and Textbook of Diagnostic Microbiology. Koneman, E.W. (Eds.), 4th Edn. B. Lippincott Co., Philadelphia, PA., 163-170

Kwakman, P.H.S., te Velde, A. A., de Boer, L., V and enbroucke-Grauls, C.M.J.E., and Zaat, S.A.J. (2011). Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS One, 6(3): ArticleIDe17709

Lee J, J.i. Y., Lee, S. and Lee, I. (2007). Effect of Saliva miltiorrhizabunge on antimicrobial activity and resistant gene regulation against methicillin-resistant Staphylococcus aureus (MRSA). Journal of Microbiology Seoul, 45: 350

Leibovici, L., Paul, M., and Andreassen, S. (2010). Balancing the benefits and costs of antibiotic drugs: the TREAT model. Clinical Microbiology and Infection. 16: 1736–1739

Maddocks, S. E., Lopez, M.S., Rowlands, R. S. and Cooper, R. A. (2012). Manuka honey inhibits the development of Streptococcus pyogenes biofilms and causes reduced expression of two fibronectin binding proteins. Microbiology, 158(Pt 3):781-90

Michelle, E.M. Campeau.,and Robin, Patel. (2014). Antibiofilm Activity of Manuka Honey in Combination with Antibiotics. International Journal of Bacteriology, 2014, Article ID 795281, 7 pages

Mohammad, R., D. Kamran, F., Jalal, S. and Jalil Dolgarri, S. (2008). Evaluating Antibacterial Activity of Iranian Honey through MIC method on some Dermal and Intestinal Pathogenic Bacteria. Med Well J, 7(4): 408-412

NCCLS (1993). Performance standards for antimicrobial disc susceptibility tests. Approved standard NCCLS publication M2-A5, Villanova, PA, USA

Patrick, Muller., Dagmar, G. Alber., Lynne, Turnbull., Ralf, C. Schlothauer., Dee, A. Carter., Cynthia, B. Whitchurch.,and Elizabeth, J. Harry. (2013). Synergism between Medihoney and Rifampicin against Methicillin-Resistant Staphylococcus aureus (MRSA). J. PLOS ONE, 8 (2): 57-79

Patton, T., Barrett, J., Brennan, J.and Moran N (2006). Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. J. Microbiolog. Meth., 64(1): 84-95

Raha, J. J. (2006). Novel antibiotic combinations against infections with almost completely resistant Pseudomonas aeruginosa and Acinetobacter species. Clinical Infectious Diseases, 43:S95–S99

Raymond, Mouokeu. S., Rosalie, Ngono. A.N., Paul, Lunga. K., Martin, Koanga. M., Alambert, Tiabou. T., Guy, Njateng. S. S. and *et al*.. (2011). Antibacterial and dermal toxicological profiles of ethyl acetate extract from Crassocephalum bauchiense (Hutch.) Milne-Redh (Asteraceae). BMC Complemen Altern Med, 11: 43

Saleem, M., Nazir, M., Ali, M.S., Hussain, H., Lee, Y.S., and *et al*.. (2010). Antimicrobial natural products: an update on future antibiotic drug candidates. Nat Prod Rep, 27, 238–254

Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U., and Molan, P. (2009). Medical honey for wound care-still the 'latest resort'. Evid Based Complement Alternat Med., 6: 165–173

Taormina, P.J., Niemira, B.A., andBeuchat, L.R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int J Food Microbiol, 69:217-225

Wagner, H., andUlrich-Merzenich, G. (2009). Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine, 16:97–110

Wilkinson, J.M., and Cavanagh, H. M. (2005). Antibacterial activity of 13 honeys against Escherichia coli and Pseudomonas aeruginosa. J Med Food, 8:100 -103

ARABIC SUMMARY

التأثيرات التآزرية للعسل والمضادات الحيوية المستخدمة عادة على البكتريا موجبة الجرام

السيد السعيد مسعود*- عبد الرحمن محمد القرشي- محمدين علي الأمين
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هدفت هذه الدراسة إلى تقييما لتأثير المثبط لكل من عسل السدر وعسل السمور منفردين وكذلك تقييم التأثيرات التآزرية لكل منهما مع 5 مضادات حيوية على البكتريا موجبة الجرام. وتعد هذه هي المرة الأولى التي يتم فيها تقييم التأثير المثبط والتأثير التآزري لعسل السمور على البكتريا. أظهرت النتائج أن كلا من عسل السدر وعسل السمور منفردين لهما تأثير مثبط على المعزولات الإكلينيكية والمعزولات المرجعية لميكروبي الاستافيلوكوكس أوريوس والاستربتوكوكس بيوجين، وكانت المعزولات المرجعية أكثر حساسية لعسل السدر بينما كانت المعزولات الإكلينيكية أكثر حساسية لعسل السمور. بينت الدراسة أن الزيادة في تركيز العسل منفردا أو مع المضادات الحيوية أدى إلى زيادة التأثير المثبط على البكتريا. وقد شوهد أعلى تأثير تآزري للعسل مع المضادات الحيويةعلى ميكروب الاستربتوكوكس بيوجين حينما تم استخدام عينتي العسل مع الأوفلوكساسين، الببراسيللين، الأموكسيسيللين + الكلافيولانيك أسيد والسلفامثوكسازول+ الترامیثوبريم. أثبتت الدراسة أن استخدام عسل السدر وعسل السمور يحسنان نشاط المضادات الحيوية ضد البكتريا موجبة الجرام.