

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

# ANTIMICROBIAL ACTIVITY OF EGYPTIAN SIDR HONEY AND ITS SYNERGISTIC ACTION WITH ANTIMICROBIAL AGENTS

Mısır Sidr Balının Antimikrobiyal Aktivitesi ve Antimikrobiyal Ajanlarla Sinerjik Etkisi

Nageh, S.M. OMRAN<sup>1</sup>, Mostafa M. M. HASSAN<sup>2\*</sup>, Mohamed F. ABDEL-RAHMAN<sup>2</sup>,  
Abd El-Aleem S.S. DESOKY<sup>1</sup>, Sayed M. HAMOUDA<sup>3</sup>

<sup>1</sup>Plant Protection Department, Faculty of Agriculture, Sohag University, EGYPT, E-posta: nagehomran@yahoo.com. ORCID No: 0009-0006- 8285- 2558, E-posta: abdelalem2011@gmail.com, ORCID No: 0009- 0003- 1738- 5189.

<sup>2</sup>Bee Research Department, Plant Protection Research Institute, Agriculture Research Center, Assiut, EGYPT, Corresponding author: E-mail: m65866236@gmail.com, ORCID No: 0000-0002-1592-0948, E-posta: m\_fathalla70@yahoo.com, ORCID No: 0000-0002-0721-9756.

<sup>3</sup>Assiut Regional Lab., Animal Health Research Institute, Agriculture Center, Assiut, EGYPT, E-posta: smhamuda60@gmail.com. ORCID No: 0000-0002-3382-5637.

Geliş Tarihi / Received: 08.09.2022

Kabul Tarihi / Accepted: 16.11.2022

DOI: 10.31467/uluaricilik.1170635

### ABSTRACT

Determine the *in vitro* antibacterial potential activity sidr honey produced in upper Egypt against five references bacterial strains (Gram positive and Gram negative strains) and its synergistic effect with some antimicrobial agents. **Material & Methods:** fifteen Sidr honey samples were collected from three Governorates in Upper Egypt. Honey samples were diluted and tested against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus* by agar dilution method. Post determination of the minimum inhibitory concentration (MIC) values, six honey samples were examined for their synergistic action with the ineffective antimicrobial agents. **Results:** *In vitro* antimicrobial sensitivity test, all bacterial strains showed multidrug resistance action against the 13 tested antimicrobial agents with high multiple antibiotic resistance (MAR) index, it was  $\geq 0.38$ . All Sidr bee honey samples showing antibacterial activity against the five tested references bacterial strains. All Sidr bee honey samples, showed better synergistic effect with all antimicrobial agents against.

**Key words:** Egyptian Sidr honey, Antimicrobial impact, Minimum inhibitory concentration, Synergistic effect

### ÖZ

Bu çalışmanın amacı, Yukarı Mısır'da üretilen Sidr balının beş referans bakteri suşuna (Gram pozitif ve Gram negatif suşlar) karşı *in vitro* antibakteriyel potansiyel aktivitesini ve bazı antimikrobiyal ajanlarla sinerjistik etkisini belirlemektir. Yukarı Mısır'daki üç valilikten on beş Sidr balı örneği toplanmıştır. Bal örnekleri seyreltilmiş ve agar seyreltme yöntemiyle *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Klebsiella pneumoniae* ve *Bacillus cereus*'a karşı test edilmiştir. Minimum inhibitör konsantrasyon (MİK) değerlerinin belirlenmesinden sonra, altı bal örneği etkisiz antimikrobiyal ajanlarla sinerjik etkileri açısından incelenmiştir. *In vitro* antimikrobiyal duyarlılık testinde, tüm bakteri suşları test edilen 13 antimikrobiyal ajana karşı yüksek çoklu antibiyotik direnci (MAR) indeksi ile çoklu ilaç direnci etkisi göstermiştir, bu değer  $\geq 0.38$ 'dir. Tüm Sidr arı balı örnekleri, test edilen beş referans bakteri suşuna karşı antibakteriyel aktivite göstermiştir. Tüm Sidr arı balı örnekleri, tüm antimikrobiyal ajanlara karşı daha iyi sinerjik etki göstermiştir.

**Anahtar kelimeler:** Mısır Sidr balı, Antimikrobiyal etki, Minimum inhibitör konsantrasyon, Sinerjistik etki

# ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

## GENİŞLETİLMİŞ ÖZET

**Giriş:** Artık dünyanın bulaşıcı hastalıklardan kurtulma umudu daha etkili ve güvenli antimikrobiyal ajanlara ulaşmaktır. Düşük bakım maliyeti ve yerel olarak bulunabilirliği bala alternatif bir antimikrobiyal tedavi olarak kullanım için değerli avantajlar sağlamaktadır. Arı balının antimikrobiyal aktivitesi fiziksel ve kimyasal özelliklerine, toplam fenol içeriğine, coğrafi bölgeye ve bitki kaynaklarına bağlıdır. Bu süre zarfında, Yukarı Mısır'da üretilen sidr balı (UESH) ticari olarak üretilmeye başlanmıştır; konu, çok ilaca dirençli (MDR) farklı bakteri suşlarına karşı antimikrobiyal aktivitesinin belirlenmesini gerektirmektedir. Tatlı bir materyal olan UESH, antimikrobiyal etkiye ve antimikrobiyal ajanlarla sinerjik etkiye sahiptir, bu nedenle bakteriyel enfeksiyon vakalarının tedavisinde tamamlayıcı ve alternatif ilaç olarak kullanılabilir.

**Amaç:** Bu çalışmanın amacı, Yukarı Mısır Sidr arı balının beş referans (3 Gram +ve ve 2 Gram -ve) bakteri suşuna karşı in vitro antibakteriyel yeteneğini ve bazı antimikrobiyal ajanlarla sinerjik etkisini belirlemektir.

**Gereç ve Yöntem:** Yukarı Mısır'daki üç farklı valilikte (Sohag, Qena ve Luxor) bulunan çeşitli arılıklardan on beş Sidr balı örneği toplanmıştır. Bal örnekleri agar difüzyon yöntemi kullanılarak 5 referans bakteri suşuna (çoklu ilaca dirençli bakteriler olarak bilinen *Staphylococcus aureus* AUMC B - 261, *Streptococcus agalactiae* AUMC B - 253, *Escherichia coli* AUMC B - 243 ve *Klebsiella pneumoniae* AUMC B - 257; ve gıda toksisitesine neden olan *Bacillus cereus* AUMC B - 100) karşı test edilmiştir. MİK değerlerinin belirlenmesinden sonra, altı bal örneği etkisiz antimikrobiyal ajanlarla sinerjik etkileri açısından incelenmiştir.

**Bulgular:** In vitro antimikrobiyal duyarlılık testinde tüm referans bakteri suşları, test edilen 13 antimikrobiyal ajana karşı çoklu antibiyotik direnci (MAR) indeksi  $\geq 0.38$  olarak yüksek çoklu ilaç direnci (MDR) göstermiştir. Tüm Sidr bal örneklerine karşı test edildiklerinde, genel ortalama  $15,16 \pm 3,77$  olmak üzere farklı MİK değerleri (%11,6 - 18,8) ile büyüme inhibisyonu elde etmişlerdir. Sohag'dan toplanan Sidr balı örnekleri, in vitro testlerde %13,6 MIC değeri gösterdiğinden en iyi antimikrobiyal etkiyi ortaya koymuş, bunu sırasıyla %14,36 ve %17,96 ile Qena ve Luxor örnekleri izlemiştir. Tüm Sidr arı balı örnekleri, tüm antimikrobiyal ajanlarla en iyi sinerjik etkiyi göstermiştir.

**Sonuç:** Çalışma, tüm Yukarı Mısır Sidr arı ballarının %11,6 ila %18,8 (v/v) arasında değişen in vitro en iyi minimum inhibitör konsantrasyon değerlerine sahip olduğu sonucuna varmıştır. Çalışılan tüm Sidr balı örneklerinin antimikrobiyal etkisi, çoklu ilaca dirençli Gram pozitif veya Gram negatif bakterilere karşı bile umut verici minimum inhibitör konsantrasyon değerlerine sahipti ve Sohag vilayetinden toplanan Sidr balı örnekleri en iyi antimikrobiyal etki göstermiştir. Ayrıca, Sidr balı örnekleri dirençli bakterilere karşı etkili olmayan antibiyotik ajanlarla sinerjik aktivite göstererek onları duyarlı hale getirmiştir. Elde edilen sonuçlar, Yukarı Mısır Sidr arı balının tüm api-terapötik kullanımlar için kullanılabilirliğini göstermiştir.

Tüm UESH örnekleri, test edilen tüm Gram pozitif veya negatif bakteriler üzerinde büyümeyi engelleyici etkiye sahiptir ve en çok test edilen antimikrobiyal ajanlarla sinerjik etki göstermiştir. Yerel olarak temin edilebilen UESH balının antibakteriyel aktivitesi, çoklu ilaca dirençli ve gıda kaynaklı bakterilere karşı terapötik ajan olarak kullanılabilir.

## INTRODUCTION

Honey antimicrobial activity is proved in ancient medicine where the primary characteristic of "Active Honey" is the presence and concentration of antibacterial compounds (Brudzynsk 2021). The difference in antibacterial activity of bee honey against different pathogens attributes to seasonality of tested honey types, difference in floral origin, physical and chemical properties, total phenol contents, geographical area as well as plant resources (Elbanna et al. 2014, Almasaudi 2021).

Determination of in vitro honey antimicrobial activity is widely documented (Hegazi 2011, Abdul-Hafeez et al. 2021) but with different potencies owing to the action of honey macro-components (sucrose, glucose) and the bioactive micro-components flavonoids (Go'miak et al. 2019), also, polyphenols that seems to contribute to the most antibacterial activity (Stagos et al. 2018), in addition to defensin (Bucekova et al. 2017) and hydrogen peroxidase and catalase enzymes (Scripcă et al. 2019), these components are acting chemically resulting in production of new created bioactive micro-components (Almasaudi 2021, Hamouda et al. 2019).

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

The high osmotic pressure and high acidity are the main two factors of antibacterial activity of undiluted honey while the activity of diluted honey is peroxide - dependent. (Zainol et al. 2013). This activity is highly complex and still remains not fully recognized which is determined by agar dilution or serial diffusion methods (Szweda 2017).

The application of antibiotics with honey yielded potential synergistic effects against microbes multidrug resistance bacterial strains even biofilm producers (Liu et al. 2018, Abdul-Hafeez et al. 2021, Almasaudi 2021). The low maintenance cost, local production and low price give honey good advantage to use as an alternative antimicrobial treatment (Mandal and Mandal 2012).

Sidr and Talah honey samples contain great potential as an antimicrobial against Gram-negative bacteria (Gram + ve) and dermatophytes regardless of the origin of the sample. Where found that Gram-negative more resistant to the tested bacteria than Gram (Owayss et al. 2019).

*In vitro* there was synergistic affect between honey and antibiotics against methicillin resistance *Staphylococcus aureus* (MRSA) and clinical isolates of *Staphylococcus aureus*. In addition, the honey and antibiotics combination stopped the appearance of antibiotics resistant *S. aureus*. The susceptibility of isolated bacterial strains revealed the synergistic effect of added honey to the antibiotic discs tested. Also, when honey was added with the antibiotic discs, there was a highly synergistic increase in the mean areas of inhibition (Abdul-Hafeez et al. 2021).

So, the present work aimed to study *in vitro* antibacterial activity sidr honey produced in upper Egypt (UESH) from different Governorates against five bacterial references strains (three Gram positive and two Gram negative strains) and their synergistic action with antimicrobial agents.

### MATERIAL AND METHODS

#### Honey Samples:

The present study was carried out through flow season of Sidr honey, during the year 2021, collected in three Governorates in Upper Egypt (Sohag, Qena & Luxor). The antimicrobial activity of selected monofloral 15 Sidr honey samples was tested against 5 reference bacterial strains.

The quantitative and qualitative evaluation of pollen grains was carried out under microscopy slide. The results of the study showed that Egyptian Sidr honey is considered a mono - floral honey with a high content of Sidr honey pollen grains with an average more than 45000 sidr pollen grains per 10 gm of honey (Louveaux et al.1978).

#### Bacterial Strains:

Five reference bacterial strains known to be pathogenic to human were chosen to evaluate the antimicrobial activity of monofloral 15 Upper Egyptian Sidr bee honey samples. The selected tested strains included pathogenic Gram-positive and Gram-negative bacteria- which were provided kindly from Assiut University Mycological Center (AUMC), Assiut, Egypt-(*Bacillus cereus* AUMC B-100, *Staphylococcus aureus* B- 261, *Streptococcus agalactiae* AUMC B- 253, *Escherichia coli* AUMC B-243 and *Klebsiella pneumoniae* AUMC B- 257 and *Bacillus cereus* AUMC B - 100; that causing food toxicity. The bacterial study carried in Assiut Regional Lab., Animal Health Researcher Institute; Agriculture Researcher Center, Egypt. All tested bacterial were used as freshly prepared to be adjusted to a 0.5 McFarland opacity standard in each bacterial inoculation all over the study to obtain approximately  $5 \times 10^5$  CFU/ml (Quinn et al. 2004).

**Antimicrobial Sensitivity Testing:** The antibiotic susceptibility of the examined isolated bacteria was evaluated to calculate multi antibiotic resistance (MAR) index of examined strains. Overnight broth cultured was compared with 0.5 McFarland standards, using the Kirby-Bauer disc spread sensitivity method as described in the National Laboratory Standards Committee guidelines (2000). The above mentioned five reference bacterial strains (*Staphylococcus aureus* B-261, *Streptococcus agalactiae* AUMC B-253, *Escherichia coli* AUMC B-243, *Klebsiella pneumoniae* AUMC B-257 and *Bacillus cereus* AUMC B-100) were tested against 13 antimicrobial agents [Cloxacillin ( CX) 1µg, Erythromycin (E) 15µg, Norfloxacin (Nor) 5µg; Cefotaxime (CTX) 30µg, Levofloxacin (Lev) 5µg, Tobramycin (TOB) 10µg; Vancomycin (VA) 30µg, Tetracycline (TE) 30µg, Gentamicin; (CN) 10µg, Ofloxacin ( Ofx) 5µg; Amoxycillin (AML) 10µg; Amoxycillin/ Clavulanic acid (AMC) 30µg; and Trimethoprim - Sulfamethoxazole (SXT) (25µg-1.25/23.75µg); Oxoid -England] For Cloxacillin inhibition zones diameter around the disc were measured after 24 and 48 h using the following

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

breakpoints: susceptible  $\geq 13$ mm and resistance  $\leq 11$ mm (Quinn et al. 2004). Multiple antibiotic resistance (MAR) index of each tested bacterial strain was calculated; to determine the MAR index that was defined as a/b, where (a) represents the number of antibiotics that isolated strain was resistant and (b) represents the number of all tested antibiotics (Subramani and Vignesh 2012).

### Honey Minimum Inhibitory Concentration (MIC)

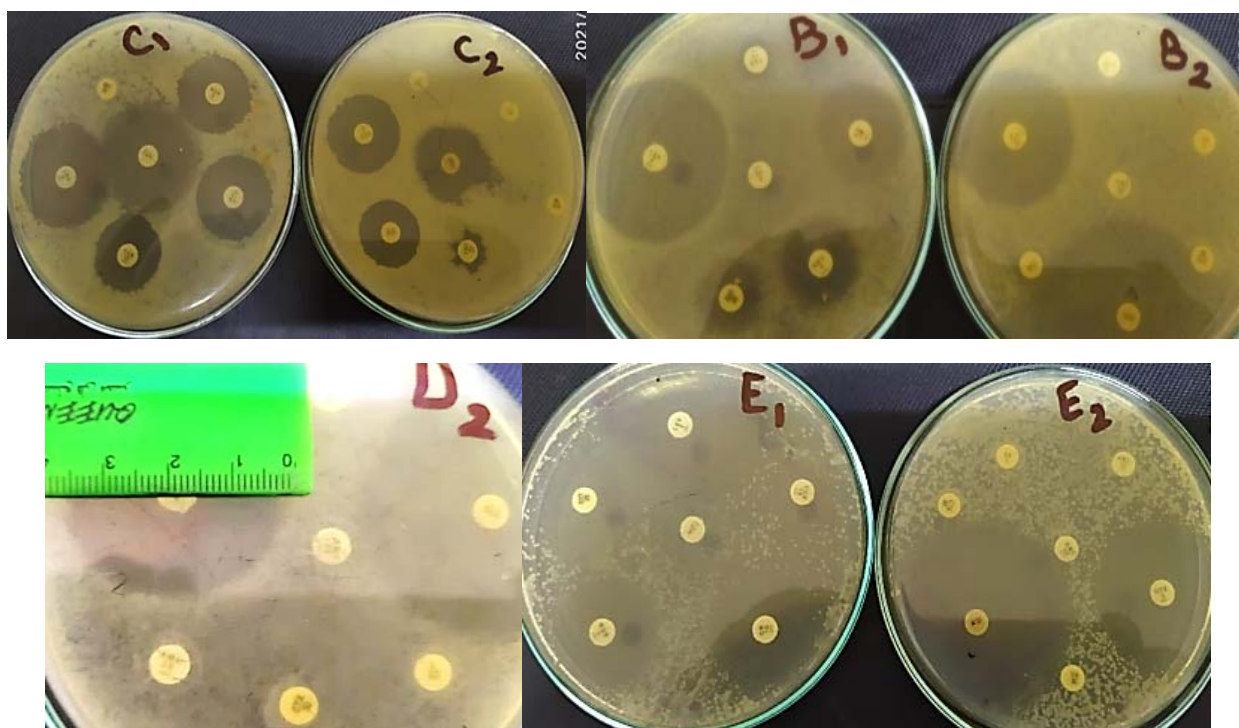
**Determination:** It was performed according to Cooper et al. 2002 method by an agar dilution method.

### Synergism between Sidr honeys and ineffective antimicrobial agents:

Synergy assay was based on described procedures by Stepanović et al. (2003), where the five strains, six Sidr honey samples and inactive antibacterial agents was evaluated. As follows, by determination of the MIC of honey, different dilutions of samples are less than 1% minimum inhibitory concentration of honey (sub-inhibitory concentration) prepared (allowing bacteria to grow).

## RESULTS

*In vitro* the present antimicrobial susceptibility testing showed that all tested strains were highly sensitive to Levofloxacin, Norfloxacin, Gentamycin, Tetracycline and Ofloxacin (100%). While the tested five reference bacterial strains were resisted Tobramycin and Vancomycin with percentage 60% followed by 40% towered Erythromycin and Trimethoprim/ Sulphamethoxazole. But the highest percentage of resistance was to Amoxycillin, Cloxacillin, Amoxycillin/ Clavulanic acid and Cefotaxime (100%); Table (1) and Plate (1) Multiple Antimicrobial resistance index (MAR) among the tested five reference bacterial strains; *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus agalactiae* showed the highest value as 0.54, while *Bacillus cereus* and *Klebsiella pneumoniae* showed lowest value 0.38; for each. Table (1) and Plate (1) the all-tested strains had very high MAR index value since MAR index value just  $\geq 0.2$  was considered high.



**Plate 1:** In Vitro antimicrobial susceptibility testing of *Escherichia coli* (B), *Klebsiella pneumoniae* (C), *Staphylococcus aureus* (D) and *Streptococcus agalactiae* (E) against 13 antimicrobial agents showing multi-drug resistance.

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

In the present study, the minimum inhibitory concentration (MIC) of Sidr bee honey samples collected from Upper Egypt and the antibacterial activity was evaluated against five reference bacterial strains, showing varying antibacterial activity against the various tested bacterial strains. Minimum inhibitory concentration of Egyptian Sidr bee honey samples, from Upper Egypt, against *Bacillus cereus*, *Escherichia coli*, *Klebsiella*

*pneumoniae*, *Staphylococcus aureus* and *Streptococcus agalactiae* showing varying antibacterial activity were 14.2±2.36, 15.0±1.6, 15.4±2.56, 15.4±2.18 and 15.8±2.27 %, respectively. The highest MIC value (15.8±2.27 %) was found against *Streptococcus agalactiae*. On the contrary, the lowest MIC value (14.2±2.36 %) was showed against *Bacillus cereus*, with overall mean 15.16±2.19%, (Table 2 & Diagram 1, 2 & Fig. 1–4).

**Table 1:** In vitro antimicrobial susceptibility test of the tested bacterial strains and multiple antibiotics resistance Index (MAR).

Bacterial strains	AMC (30 mcg)	CTX (30 mcg)	CX (1 mcg)	AML (10 mcg)	OFX (5 mcg)	VA (30 mcg)	TE (30 mcg)	TOB (30 mcg)	CN (10 mcg)	NOR (5 Mcg)	SXT (1.25 /23.75 mcg)	LEV (5 mcg)	E (15 mcg)	MAR (multiple antibiotics Resistance Index)
	The diameter of inhibition zone in mm													
<i>Bacillus cereus</i>	14	Zero	Zero	11	25	20	25	19	24	21	Zero	28	30	SXT, AML, CX, CTX, AMC(0.38)
	R	R	R	R	S	S	S	S	S	S	R	S	S	
<i>Escherichia coli</i>	Zero	Zero	Zero	Zero	30	8	>30	11	25	21	10	40	25	SXT, TOP, VA, AML, CX, CTX, AMC, (0.54)
	R	R	R	R	S	R	S	R	S	S	R	S	S	
<i>Klebsiella pneumoniae</i>	10	Zero	Zero	Zero	25	20	24	20	24	25	Zero	30	25	SXT, AML, CX, CTX, AMC (0.38)
	R	R	R	R	S	S	S	S	S	S	R	S	S	
<i>Staphylococcus aureus</i>	Zero	Zero	Zero	Zero	35	Zero	>30	12	25	25	22	40	Zero	E, TOP, VA, AML, CX, CTX, AMC, (0.54)
	R	R	R	R	S	R	S	R	S	S	S	S	R	
<i>Streptococcus agalactiae</i>	Zero	Zero	Zero	Zero	>33	Zero	>40	12	23	23	22	>30	Zero	E, TOP, VA, AML, CX, CTX, AMC, (0.54)
	R	R	R	R	S	R	S	R	S	S	S	S	R	

AMC= Amoxicillin/Clavulanic acid, CTX= Cefotaxime, CX= Cloxacillin, AML= Amoxicillin, OFX= Ofloxacin, VA=Vancomycin, TE= Tetracycline, E= Erythromycin, LEV= Levofloxacin, NOR= Norfloxacin, CN=Gentamicin, TOB=Tobramycin, SXT=Trimethoprim/Sulfamethoxa

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

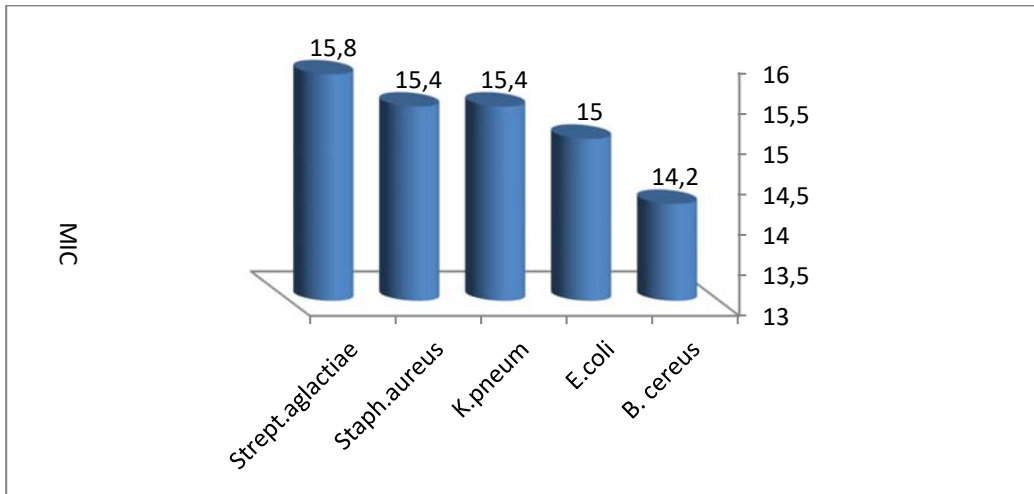


Diagram (1): Percentage of the minimum inhibitory concentration (MIC) of Upper Egyptian Sidr bee honey samples against the tested bacterial strains.

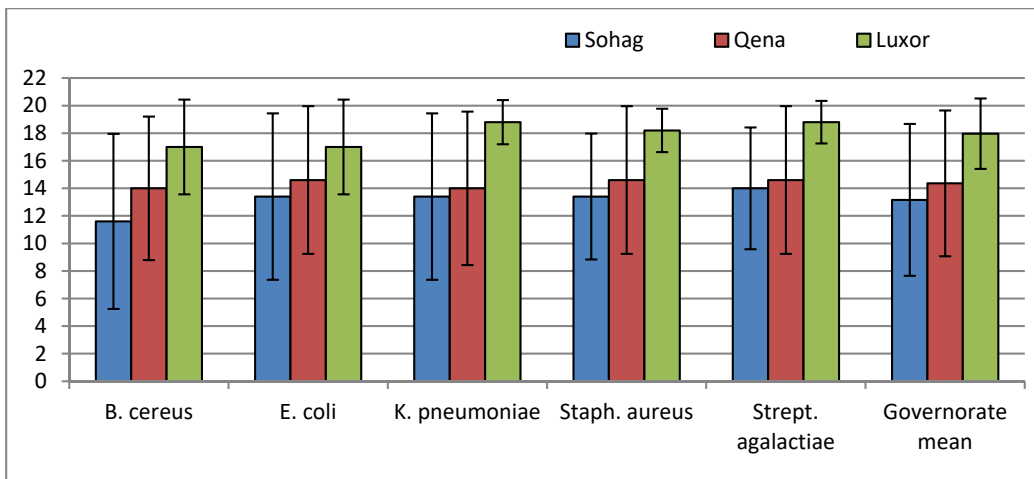


Diagram (2): Percentage of the minimum inhibitory concentration (MIC %) of Sidr honey samples from different locations of Upper Egypt against the tested bacterial strains.

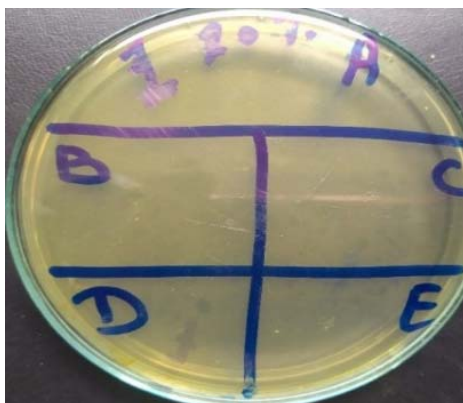


Figure 1: In Vitro honey (20%) showing no growth against all five reference strains



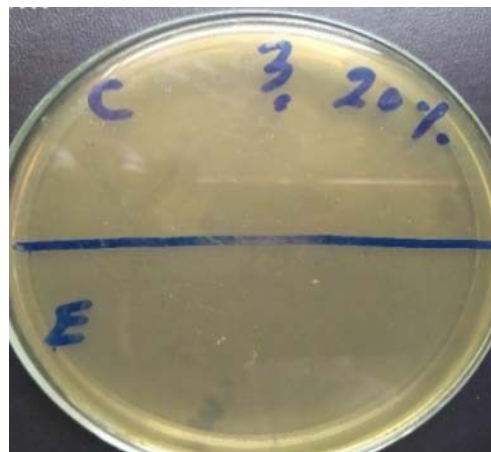
Figure 2: In Vitro honey (11%) showing no growth against *Staph. aureus* (D) and *Strept. agalactiae* (E).



## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE



**Figure 3:** In Vitro honey at (10%) all reference strains showing growth



**Figure 4:** In Vitro honey (20%) showing no growth all five against *K. pneumonia* (C), and *Strept. agalactiae* (E)

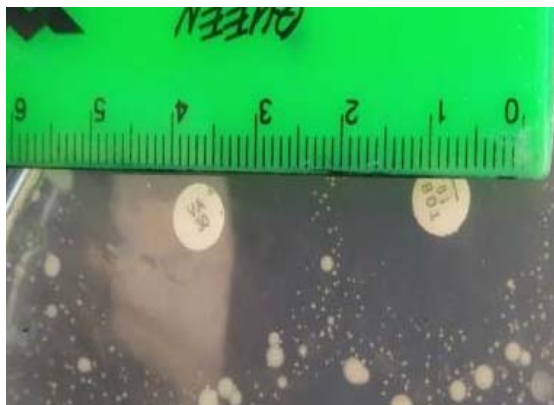
**Table 2.** Percentage of the minimum inhibitory concentration (MIC %) of Sidr honey samples from different locations of Upper Egypt against the tested bacterial strains.

Location	MIC percentage v/v against different bacterial strains					Mean of location
	<i>Bacillus cereus</i> (B.100)	<i>Escherichia coli</i> (B243)	<i>Klebsiella pneumonia</i> (B257)	<i>Staphylococcus aureus</i> (B251)	<i>Streptococcus</i> (B253)	
Sohag	11.6±0.24	13.4±0.42	13.4±0.23	13.4±0.19	14.0±0.26	13.6±0.225 C
Qena	14.0±0.33	14.6±0.12	14.0±0.17	14.6±0.39	14.6±0.17	14.36±0.375 B
Luxor	17.0±0.44	17.0±0.17	18.8±0.09	18.2±0.33	18.8±0.12	17.96±0.872 A
Mean of bacterial strain	14.2±2.36 D	15.0±1.6 C	15.4±2.56 B	15.4±2.18 B	15.8±2.27 A	15.16±2.19
Range	11.6-17.0	13.4-17.0	13.0-18.8	13.4-18.2	14.0-18.8	-

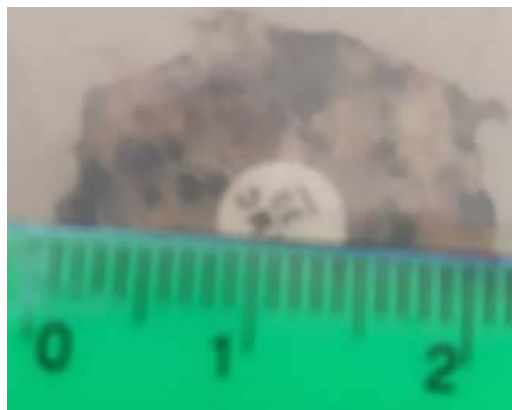
In this study, the high antibacterial activity of honey samples against all test strains support the assessment of synergistic action of honey and antibiotics. Honey interacts synergistically with antibiotics at sub-lethal concentrations (sub-MIC), so the present work tested this activity for six brands of Sidr honey. with the ineffective antimicrobial agents.

All Sidr honey brands, in the present study, showed best synergistic action with different antimicrobial agents against *Staphylococcus aureus* (B 261), *Klebsiella pneumoniae* (B 257), *Bacillus cereus* (B100) and *Streptococcus agalactiae* (B 253), Table (3) Fig. (5-7).

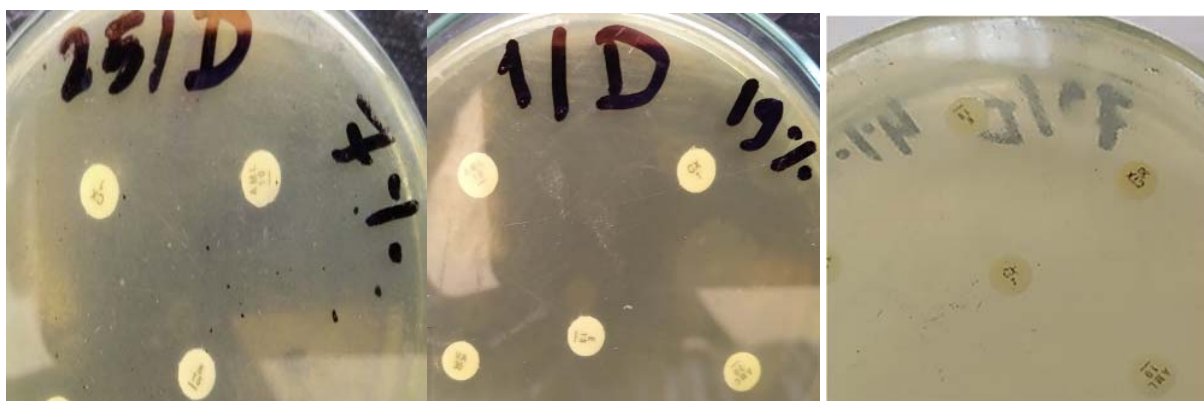
## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE



**Figure 5:** Synergistic action of Sidr honey TOB and VA against *E. coli*.



**Figure 6:** Synergistic action of Sidr honey with AMC against *K. pneumonia*.



**Figure 7:** Different types of Sidr honeys (A, B and C) restored methicillin resistant *S. aureus* (MRSA) sensitive to Cloxacillin (MSSA).

**Table 3:** Synergistic action of Sidr bee honey and the ineffective antimicrobial agents against both *Staph. aureus* (B 261) and *Strept. agalactiae* (B 253)

Antimicrobial agents	Breakpoint $\geq$ mm	zone of inhibition of antimicrobial disc only	zone of inhibition of tested Sidr honey with antimicrobial disc in mm					
			I	II	III	IV	V	VI
Amoxycillin	29 mm	Zero mm	33 mm	35 mm	33 mm	33 mm	33 mm	35 mm
Amoxycillin / Clavulanic	20 mm	Zero mm	25 mm	22 mm	24 mm	22 mm	23 mm	22 mm
Vancomycin	12 mm	Zero mm	19 mm	26 mm	20 mm	19 mm	24 mm	25 mm
Cloxacillin	13 mm	Zero mm	16 mm	15 mm	18 mm	17mm	15 mm	18 mm
Tobramycin	15 mm	12 mm	23 mm	20 mm	18 mm	21 mm	19 mm	22 mm
Erythromycin	23 mm	Zero mm	26 mm	28 mm	30 mm	28 mm	27 mm	29 mm
Cefotaxime	23 mm	zero mm	25 mm	27 mm	29 mm	25 mm	29 mm	30 mm



## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

### DISCUSSION

In the present study, the tested bacterial strains showed strong multidrug resistance action (Table 1 & Plate 1) against different antimicrobial groups;  $\beta$ -lactam (amoxicillin, cloxacillin, amoxicillin/clavulanic acid) or against the third-generation cephalosporin (cefotaxime) showed resistance 100% resulting that the tested *Staphylococcus aureus* B – 261 was methicillin resistance *Staphylococcus aureus* (MRSA). But against quinolone compound (tobramycin) and glycopeptide (vancomycin) showed resistance 60%, while against macrolide (erythromycin) and sulfonamides (Trimethoprim - Sulfamethoxazole) was only 40% resistance.

But all tested strains were highly sensitive to Levofloxacin, Norfloxacin, Gentamycin, Tetracycline and Ofloxacin (100%), (Table 1). These obtained resistance results were with high value of multiple antibiotic resistance (MAR) index as it was  $\geq 0.38$  [0.38- 0.54] since MAR index  $\geq 0.2$  is considered high (Subramani and Vignesh 2012). These results go in parallel with that obtained (Abdul-Hafeez et al. 2021, Sayed et al. 2011).

But fortunately, there is no bacterial resistance against honey as that antibiotics (Blair et al. 2009; Cooper et al. 2010). In the present study, the minimum inhibitory concentration (MIC) of Sidr honey samples collected from Upper Egypt showing varying antibacterial activity against the various tested bacterial strains.

All tested Upper Egyptian Sidr honey (UESH) samples, in present work, have different potencies of antibacterial effects against all five reference bacterial strains with a percentage of minimum inhibitory concentration. (MIC%) values were 13.6 - 17.96% (v/v), with the mean percentage minimum inhibitory concentration (MIC%) values as 15.16% (v/v), (Table 2 & Diagram 1); Generally, the percentage of the minimum inhibitory concentration of Upper Egyptian sider honey samples against *Bacillus cereus* AUMC B- 100, *Escherichia coli* AUMC B- 243 and *Klebsiella pneumoniae* AUMC B- 257 *Staphylococcus aureus* B- 261, and *Streptococcus agalactiae* AUMC B- 253, were 14.2 $\pm$ 5.97, 15 $\pm$ 5.18, 15.4 $\pm$ 5.32, 15.4 $\pm$ 4.56 and 15.8 $\pm$ 4.56%, respectively. The highest MIC% value (15.8 $\pm$ 4.56%) was found against *Streptococcus agalactiae*. While the lowest MIC value (14.2 $\pm$ 5.97%) was showed against *Bacillus cereus* (Table 2, Fig. 1 - 4 & Diagram 1). In the present work,

Sidr honey showed broad-spectrum antimicrobial activity against Gram+ve and Gram-ve bacteria, which is agreement With previous findings where different types of honey from diverse botanical origins were reported with widespread activity against Gram+ve and Gram-ve bacteria (Irish et al. 2011, Hegazi & Abd Allah 2012, Elbanna et al. 2014, Almasaudi et al. 2017). honey samples collected from Sohag were having goodly antibacterial efficacy against the tested bacterial strains revealed MIC% was 13.6 % followed by samples of Qena and Luxor as 14.36 & 17.96 %, respectively (Table 2 & Diagram 2).

The antibacterial potency differences among honey samples could be attributed to the natural variations the different geographical locations and floral sources of nectar (Alzahrani et al. 2012, Da Silva et al. 2016). The difference in antimicrobial potency between different types of honey can be more than 100-fold, depending on its geographical, seasonal and botanical source (Molan and Cooper 2000). Bacterial strains showed differential sensitivity as Gram-positive bacteria were more sensitive than Gram-negative bacteria (Owayss et al, 2019). Gram-positive and Gram-negative bacteria were inhibited by 5-10 and 10 to 20 % (w/v), respectively (Eman and Mohammed 2011).

These results are According to other authors, Sidr honey has been used as an antimicrobial agent. The average MIC values of Sidr honey was 15%, 20%, 10% & 20% (v/v) (Alqurashi et al. 2013, Almasaudi et al. 2017, Mohammed & Jayashankar 2020), respectively. The present obtained MIC values of Upper Egyptian Sidr bee honey were much less than MIC values of either Egyptian Sidr Honey recorded formerly as 20% (Hamouda et al. 2019) or the fully studied Saudi Sidr Honey as 20% (Hegazi 2011, Almasaudi et al. 2017, Hegazi et al. 2017).

Sidr honey possessed MIC value against *Streptococcus pyogenes* 20% (w/v), as Sidr honey is effective at inducing lysis of bacterial cell and identifies targets genes, at the genetic level (Mohammad et al. 2021). While the another study showed high MIC that at a concentration 50% (w/v), where *Staphylococcus aureus* ATCC 29213 was susceptibility to two out of the three Sidr honey types tested, while *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27453 were completely unaffected by the three Sidr honeys tested (Dash et al. 2016). Also, Sidr honey sample showing MIC on *Staphylococcus heamolyticus* more

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

than 25% v/v (Mohammed et al. 2016). Nigerian honey at concentration 40% v/v gave better antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* (Braide et al. 2012).

Recent study of Saudi Sidr Honey showed higher MIC value as 33% w/v. Saudi Talh and Sidr honey The water-diluted honeys caused an increase in antimicrobial activity compared to the undiluted natural honey. (Owayss et al. 2019). Consequently, MIC of the Upper Egyptian Sidr bee honey revealed the best *in vitro* antimicrobial action of all available studied Sidr honey.

The potential therapeutic agent of honey is increasingly due to its antibacterial activity as the most researched biological property of honey through the influence of its phytochemical contents that vary geographically or seasonally. Sider honey has several antibacterial components and property is likely to explain why, unlike antibiotics, it does not induce resistance in bacteria. Commercial development of new classes of antibiotics has been diminished and few pharmaceutical companies still active in this area (European Center for Disease Prevention and Control 2009).

Honey interacts synergistically with antibiotics at sub-lethal (sub-MIC) concentrations (Masoud et al. 2015, Brown et al. 2020), so the present work tested this activity of six Upper Egyptian Sidr bee honey samples with the eight ineffective antimicrobial agents. All tested Upper Egyptian Sidr bee honey samples showed synergistic effect with different antimicrobial agents against the five tested bacterial strains and these resistant bacteria become sensitive (Table 3 & Fig. 5 & 6); and the best synergistic action was against *Staphylococcus aureus* (B 261) and *Streptococcus agalactiae* (B 253). Meanwhile, all tested Sidr honey samples restored *Staphylococcus aureus* sensitive to cloxacillin, Table (3) and Fig. (7).

It was documented that manuka (Jenkins and Cooper 2012, Müller et al. 2013) or Egyptian fennel honey (Abdul-Hafeez et al. 2021) restored methicillin resistant *Staphylococcus aureus* (MRSA) to methicillin sensitive *Staphylococcus aureus* (MSSA) since honey interacts synergistically with antibiotics at sub-lethal (sub-MIC) indicated by significant decline in minimum inhibitory concentration or sub-lethal concentration (Enany et al. 2018). This action is obtained by the down regulation of the honey on

methicillin resistant *Staphylococcus aureus* (MRSA) accessory gene regulator genes (*agr B*, *agr C*, *agr D*) responsible for virulence (Jenkins and Cooper 2012) and *cid B* gene responsible for bacterial cell division and reduced expression of *mecR1* (responsible for methicillin resistance) (Jenkins et al. 2014) and decreased transcription of the MRSA-specific penicillin binding protein (PBP2A) that has markedly reduced affinity to  $\beta$ -lactams compared to endogenous *Staphylococcus aureus* PBP enzymes (Liu et al. 2015). But, in the present study, *Escherichia coli* (B 243) and *Klebsiella pneumoniae* (B257) still resistant the trimethoprim – sulfamethoxazole and sub-MIC of one Upper Egyptian Sidr bee honey (UESH) sample and *Bacillus cereus* (B100) still resistant to two samples It was evident that both the antimicrobial strength and fairness brands of honey had variable synergistic activity against *Escherichia coli*, *Klebsiella pneumonia* and *Streptococcus agalactiae* (Abdul-Hafeez et al. 2021). These findings provide a strong basis for the use of Upper Egyptian Sidr honey in treatment of bacterial infections. In addition of mixing of honey and antibiotics have synergistic activity against biofilms producing bacteria. The micro-organisms may not develop resistance against honey in the same way as they develop for other commonly used antimicrobial agents. All these features may make the honey a promising alternative to the commonly used antibiotics.

### Conclusion:

The study concluded that all Upper Egyptian Sidr bee honey have the best minimum inhibitory concentration values *in vitro* ranged from 11.6 to 18.8% (v/v). Antimicrobial action of all available studied Sidr honey samples even against multi- drug resistant Gram- positive or Gram- negative bacteria was with promising minimum inhibitory concentration values, where Sidr honey samples collected from Sohag governorate showed the best antimicrobial action. Moreover, Sidr honey samples achieved synergistic activity with the non-effective antibiotic agents against resistant bacteria restoring them sensitive. The obtained results recommended that Upper Egyptian Sidr bee honey can be used for all api-therapeutic usage.

**Authors' contributions:** The authors contributed equally in the study. They designed, performed, analyzed the data, wrote and revised the manuscript.

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

**Conflict of interest:** Authors declare that they have no any conflict of interests to be reported.

**Data availability:** All data and materials used and/or analyzed during the current study are available in this manuscript.

**Ethical issue:** Not applicable because this study on honey and not animals or humans

**Source of Finance:** Not applicable because there is no funding source for this study.

### REFERENCES

- Abdul-Hafeez, MM., Hamouda, SM and Abd El Rahman, MF. Synergistic antimicrobial effect of Egyptian honey with antibiotics and its capability to restore MRSA sensitive to oxacillin. *International Journal of Science and Research*. 2021; 10 (3):1389-1395. DOI: 10.21275/SR21313001535.
- Almasaudi, BS, Alaa, AM, Al-Nahari, EI, Sayed M. Antimicrobial effect of different types of honey on *Staphylococcus aureus*. *Saudi Journal of Biological Sciences*. 2017; 24:1255–1261.
- Almasaudi, BS. The antibacterial activities of honey. *Saudi Journal of Biological Sciences*. 2021;28: 2188–2196. doi: 10.1016/j.sjbs.2020.10.017.
- Alqurashi, AM, Masoud, EA, Alamin, MA. Antibacterial activity of Saudi honey against Gram negative bacteria. *J. of Microbiology and Antimicrobials*. 2013; 5(1):1- 5. DOI: 10.5897/JMA2012.0235.
- Alzahrani, HA, Alsabehi R, Boukraâ L, Abdellah F, Bellik Y. Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. *Molecules*. 2012; 17: 10540-10549. doi: 10.3390/molecules170910540.
- Blair, S, Cokcetin, N, Harry E, Carter, D. The unusual antibacterial activity of medical-grade *Leptospermum* honey: antibacterial spectrum, resistance and transcriptome analysis. *European journal of clinical microbiology & infectious diseases*. 2009; 28: 1199–1208. doi: 10.1007/s10096-009-0763.
- Braide, W, Oranusi, S, Akaluka, C. Antibacterial efficacy of crude and diluted honey on four wound isolates. *Global Advanced Research J. of Microbiology*. 2012; 1(1): 1–4.
- Brown, HL, Georgie, M, Matthew, D. Antibacterial and antivirulence activity of Manuka Honey against genetically diverse *Staphylococcus pseudintermedius* strains. *Applied and Environmental Microbiology*. 2020; 86 (20): e01768-20.
- Brudzynsk, K. Honey as an Ecological Reservoir of Antibacterial Compounds Produced by Antagonistic Microbial Interactions in Plant Nectars, Honey and Honey Bee. *Antibiotics*. 2021; 10: 551. doi: 10.3390/antibiotics10050551.
- Bucekova, M, Martin S, Ivana V. Bee-derived antibacterial peptide, efensing-1, promotes wound reepithelialisation *in vitro* and *in vivo*. *Scientific Reports*. 2017; 7: 7340. doi:10.1038/s41598-017-07494-0.
- Cooper, R, Jenkins, L, Henriques, AFM, Duggan, R, Burton, N. Absence of bacterial resistance to medical-grade manuka honey. *European j. of clinical microbiology & infectious diseases*. 2010; 1–5. doi: 10.1007/s10096-010-0992.
- Cooper, RA, Molan, PC, Harding, KG. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *J. Appl. Microbiol*. 2002; 93(5): 857-863. doi:org/10.1046/i.1365-2672.2002.01761.x
- Da Silva, PM, Gauche, C, Gonzaga, LV, Costa, ACO, Fett R. “Honey: Chemical Composition, Stability and Authenticity” in *Food Chemistry*. 2016; 196: 309-323. doi: 10.1016/j.foodchem.2015.09.051.
- Dash, N, Debadatta, P, Mansour, Al-Zarouni. Antimicrobial Effect of Honey from the Arabian Gulf Region against Bacterial Isolates from Pus and Wound swabs. *Advances in Microbiology*. 2016; 6: 745-752. DOI: 10.4236/aim.2016.610073
- Elbanna, K, Attalla, K, Elbadry, M, Abdeltawab, A, Gamal-Eldin, H, Ramadan, MF. Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. *Asian Pacific Journal of Tropical Disease*. 2014; 4(3): 194–200. doi: 10.1016/S2222-1808(14)60504-1.

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

- Eman, MH, Mohammed, MS. Shaoka and Sidr honeys surpass in their antibacterial activity local and imported honeys available in Saudi markets against pathogenic and food spoilage bacteria. *Australian Journal of Basic and Applied Sciences*. 2011; 5(4): 187-191.
- Enany, M E, Algammal, AM, Shagar, GI. Molecular typing and evaluation of Sidr honey inhibitory effect on virulence genes of MRSA strains isolated from catfish in Egypt. *Pak. J. Pharm. Sci*. 2018; 31, (5):1865-1870.
- European Center for Disease Prevention and Control (ECDC). The bacterial challenge: time to react. Stockholm: European Center for Disease Prevention and Control. 2009.
- Go'rnjak, I, Rafał, B, Jarosław, K. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochem Rev*. 2019; 18:241–272. doi:10.1007/s11101-018-9591.
- Hamouda, SM, Heba, MMK, Mahmoud, A, Magda, MA, Haroun, AY. Apitherapy of Septic Metacarpal and Metatarsal Wounds (An Experimental Study on Donkeys). *Clinical Medicine Research*. 2019; 8(4): 77-84. doi:10.11648/j.cmr.20190804.12.
- Hegazi, AG, Abd Allah, FM. Antimicrobial activity of different Saudi Arabia honeys. *Global Veterinaria*. 2012; 9(1): 53–59.
- Hegazi, AG, Al Guthami, FM, Al Gethami, AFM. Potential antibacterial activity of some Saudi Arabia honey. *Veterinary World*. 2017; 10(2): 233-237. DOI:10.14202/vetworld.2017.233-237.
- Hegazi, AG. Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. *Res J Microbiol*. 2011; 6:488–495. doi: 10.17311/jm.2011.488.495.
- Irish, J, Blair, S, Carter, DA. The antibacterial activity of honey derived from Australian flora. *PLoS ONE*. 2011; 6(3): e18229. <https://doi.org/10.1371/journal.pone.0018229>.
- Jaktaji, PR, Ghalamfarsa, F. Antibacterial activity of honeys and potential synergism of honeys with antibiotics and alkaloid extract of *Sophora alopecuroides* plant against antibiotic-resistant *Escherichia coli* mutant. *Iran J Basic Med Sci*. 2021; 24(5):623-628. doi: 10.22038/ijbms.2021.54224.12179.
- Jenkins, R, Burton, N, Cooper, R. Proteomic and genomic analysis of methicillin resistant *Staphylococcus aureus* (MRSA) exposed to manuka honey in vitro demonstrated down-regulation of virulence markers. *J. Antimicrob. Chemother*. 2014; 9(3):603-615. doi: 0.1093/jac/dkt430.
- Jenkins, RE, Cooper, R. Synergy between oxacillin and manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. *J. Antimicrob. Chemother*. 2012; 67:1405–1407. DOI:10.1093/jac/dks071.
- Liu, M, Lu, J, Müller, P. Antibiotic specific differences in the response of *Staphylococcus aureus* to treatment with antimicrobials combined with manuka honey. *Frontiers in microbiology*. 2015; 5 (Article 779):1-9. doi:10.3389/fmicb.00779.
- Liu, MY, Nural C, Jing L. Rifampicin - Manuka honey combinations are superior to other antibiotic-Manuka honey combinations in eradicating *Staphylococcus aureus* Biofilms. *FrontiersMicrobiol*. 2018; 8(11):1-12. doi.org/10.3389/fmicb.2017.02653.
- Louveaux J, Maurizio A. and Vorwohl, G. Methods of melissopalynology. *Bee World*. 1978; 59:139-157.
- Mandal, MD, Mandal, S. Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*. 2012; 1(2): 154-160.
- Masoud, EA, Alqurashi, AM, Alamin, AA. Synergistic effects of honeys and commonly used antibiotics on Gram Positive Bacteria. *Egypt. Acad. J. Biolog. Sci*. 2015; 7(1): 101–109. DOI:10.21608/eajbsg.2015.16491.
- Mohammad, A, Al-Kafaween, MHA, Hamid ANA. Potential antibacterial activity of Yemeni Sidr honey against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. *Anti-Infective Agents in Medicinal Chemistry*. 2021;19(4):1-15. DOI:10.2174/2211352519666210319100204.
- Mohammed, AAA, Sailh, MK, Ali AF, Nusaybah, KHS. *In Vitro* antibacterial activity of some natural and trade iraqi honey against MRSA *Staphylococcus Heamolyticus* isolated from some burned patients in Misan City. *American*

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

- Journal of Microbiological Research. 2016; 4(5): 159-163. DOI:10.12691/AJMR-4-5-6.
- Mohammed, AS, Jayashankar, M. Evaluation of antibacterial activity of some Indian and Yemeni honey against few bacterial isolates from human patients. *Egypt. J. Microbiol.* 2020; 55: 21-28. doi: 10.21608/EJM.2020.1135.
- Molan, PC, Cooper, RA. Honey and sugar as a dressing for wounds and ulcers. *Trop. Doct.* 2000; 30:249-250. doi: 10.1177/004947550003000101.
- Müller, P, Alber, DG, Turnbull, L. Synergism between Medihoney and Rifampicin against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *PLOS ONE.* 2013; 8(2): doi: 10.1371/journal.pone.0057679.
- National Committee for Laboratory Standards (NCCLS) 2000. Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard M2 – A7, M100 – S10. PA.
- Owayss AA, Khaled E, Javaid I. In vitro antimicrobial activities of Saudi honeys originating from *Ziziphus spina-christi* L. and *Acacia gerrardii* Benth. *Trees. Food Sci. Nutr.* 2019; 8:390–401. doi: org/10.1002/fsn3.1320.
- Quinn PJ, Carter ME, Markey B, Carter GR. *Bacteriology: Clinical veterinary microbiology.* 2004; (6<sup>th</sup>Ed.) Mosby Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.
- Sayed, MS, Maryam FM, El Berbawy SM. Bacteriological studies on sub-clinical mastitis in cows and buffaloes with trails for its treatment. *Assiut Veterinary Medical Journal.* 2011; 57, (129):185-200. doi:10.21608/AVMJ.2011.174542.
- Scripcă, LA, Liliana N, Sonia A. Comparison of physicochemical, microbiological properties and bioactive compounds content of grassland honey and other floral origin honeys. *Molecules.* 2019; 24:1-17, 2932. doi:10.3390/molecules24162932.
- Stagos, D, Soultiotis, N, Tsadila, C. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *International Journal of Molecular Medicine.* 2018; 42(2):726–734. doi:10.3892/ijmm.2018.3656.
- Stepanović S, Antić N, Dakić I. *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microbiol. Res.* 2003; 158(4):353-357. doi:10.1078/0944-5013-00215.
- Subramani, S, Vignesh, S. MAR index study and MDR character analysis of a few golden Staph isolates. *Asian Journal of Pharmacy and Life Science.* 2012; 2(2).
- Szweda, P. Antimicrobial Activity of Honey. *Honey Analysis.* 2017; pp. 215-232. doi:10.1155/2019/2464507.
- Zainol, MI, Kamaruddin, MY, Mohd, YMY. Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine.* 2013; 13:129.