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Study of Antibacterial, Antioxidant Activity and Biochemical Parameters of Different Honey Samples

Jian Lateif Hussein[1], Akhter Ahmed Ahmed*[2], Dotsha Jaleel Raheem[1]
[1]*Department of Chemistry, College of Science, Salahaddin University- Erbil, Iraq*
[2]*Department of Biology, College of Science, Salahaddin University- Erbil, Iraq*

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Abstract

The present study was undertaken to determine and compare the antibacterial and biochemical characteristics of honey samples from Kurdistan region in Iraq and Arabian Gulf region. Sixteen honey samples of mixed floral origins from both regions were analysed and compared. Antibacterial activity of the honey samples was investigated against five clinical pathogenic bacteria: *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* by agar dilution method. Both sample sets showed differential biochemical characteristics and potential functional properties such as antioxidant and antimicrobial properties. All measured parameters were within accepted ranges. However, significant differences were found in mean±SE levels of conductivity and pH, diastase activity, TPC and DPPH activity between the two sample groups with KRI samples showing more desirable qualities. Other parameters such as water content, total acidity, total and reducing sugars, sucrose and HMF showed no significant difference. The results of antibacterial activity indicated that KRI honey is more effective than AG honey against the tested pathogenic bacteria.

Keywords: Antibacterial activity, total phenolic content, diastase, honey quality.

دراسة الفعالية المضادة للبكتريا و ضد الاكسدة والخواص الكيموحيوية لمجموعة من نماذج العسل المختلفة

ژيان لطيف حسين[1]، اختر احمد احمد*[2]، دۆتشا جليل رحيم[1]
[1]قسم الكيمياء، كلية العلوم، جامعة صلاح الدين ، أربيل، العراق
[2]قسم علوم الحياة، كلية العلوم، جامعة صلاح الدين ، أربيل، العراق

الخلاصه

تم أجراء هذه الدراسة لغرض تقدير الفعالية المضادة للبكتريا و الخواص الكيموحيوية لنماذج عسل عسل من اقليم كردستان العراق و مقارنتها مع نماذج من منطقة الخليج العربي. تضمنت الدراسة تحليل و مقارنة ستة عشر نموذجا من العسل من اصول نباتية مختلطة من كل من المنطقتين. تمت دراسة الفعالية المضادة للبكتريا لعينات العسل ضد خمس انواع بكتيرية مرضية و هي *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,* و *Staphylococcus*

*Email: akhter.ahmed@su.edu.krd

*aureus*بطريقة تخفيف الاكار. اظهرت كلتا المجموعتين صفات كيموحيوية متفاوتة و اختلاف في الفعالية المضادة للاكسدة و الفعالية الحيوية. بينت النتائج انه رغم كون الخصائص التي تمت دراستها ضمن الحدود المسموحة لكلتا المجموعتين بصورة عامة، الا أنه وجدت فروق معنوية واضحة بينهما في معدلات التوصيلية الكهربائية و الأس الهيدروجيني، فعالية أنزيم الدايستيز، المحتوى الكلي للمركبات الفينولية و فعالية تثبيط الـ DPPH بحيث كانت هذه الخصائص النوعية أعلى في النماذج العراقية. و لم تظهر معدلات المحتوى المائي، الحامضية الكلية، نسبة السكريات الكلية و المختزلة، السكروز و الـ HMF اي فروق معنوية بين المجموعتين. كما و اظهرت نتائج الفعالية الحيوية كون العسل العراقي اكثر تاثيرا على الانواع البكتيرية المرضية قيد الدراسة

1. Introduction

An important property of honey is its stability at room temperature and not requiring preservation or refrigeration. Due to its richness in a range of phenolic acids and flavonoids, honey has a remarkable antibacterial and antioxidant activity [1]. Its water content is preferred to be below 20% and its pH value around 3.9. Honey is 25% sweeter than sucrose and it is comprised primarily of carbohydrates that make up about 95-97% of its dry weight [2]. Additionally, other compounds such as organic acids, various vitamins, amino acids and minerals are also present in appreciable amounts [3]. The most abundant sugars of honey are fructose, glucose and sucrose, which play a main role in its biophysical effects [4]. Organic acids present in honey can be formed by the oxidative action of enzymes on sugars as in the formation of gluconic acid from glucose. A range of other acids are also present in small amounts that give honey its characteristic acidic pH ranging between 3.2 and 4.5. In addition to glucose oxidase, diastase and catalase are some of the main enzymes of honey [5].

Two main aspects of honey biological activity are its antioxidant and antibacterial powers. Although, the natural antioxidants function in human body is not fully implied, research has demonstrated the effects of natural honey in many processes including reaction with highly reactive oxygen species and free radicals. Damage caused by these compounds contributes to the development of cancer and other diseases [6]. The power of honey to act as antioxidant is correlated with high content of phenolic compounds which in turn is correlated with higher radical scavenging activity of a given sample. Other compounds in the chemical make-up of honey can also contribute to its antioxidant activity [7].

Honey is a potent antimicrobial agent with an extensive array of effects. Its efficiency in this regard can be linked to its content of sugars, phenolic compounds, 1,2-dicarbonyl compounds and hydrogen peroxide. These constituents work together, allowing honey to be powerful against different microorganisms including multidrug resistant (MDR) bacteria and reduce their resistance to antibiotics. Other factors that add to the antimicrobial potency and effectiveness of honey are the types and botanical sources of honey produced, bee health and honey processing techniques [8].

The present study aimed to investigate and compare the quality of local honey in honey from Kurdistan region/Iraq (KRI) with commercial honey samples from **Arab Gulf (AG) countries** in terms of **biochemical parameters,** total phenolic compounds (TPC)**, antioxidant and antibacterial activity.**

2. Materials and Methods

2.1. Collection of samples

The experimental material consisted of honey of mixed floral origins. Total of 32 samples

were studied, 16 from local apiaries located in KRI and 16 were commercially available samples from AG region.

2.2. Chemicals and instruments

All reagents and chemicals were of analytical grade. The honey samples were subjected to a number of biochemical tests, TPC, antioxidant and antibacterial activity as follows:

2.3. Biochemical tests

2.3.1. Water content and electrical conductivity

Water content was determined by measuring refractive index (RI) of honey samples using Abbe refractometer. Water content values corresponding to RI measurements were calculated from tables and variation in temperature were corrected. For measuring electrical conductance, conductivity of a 20% (w/v) honey solution in distilled water was measured using DDS-2230 conductometer. The results were expressed in µS/cm. Both parameters were determined according to Bogdanov *et al* [9].

2.3.2. Hydroxymethylfurfural (HMF)

Analysis of HMF was based on the original method by [10]. Briefly 10% solutions of honey samples containing 0.5 mL of each of Carrez I and Carrez II solutions were prepared and filtered. The basic principle of this method involves determination of HMF by measuring absorbance of honey solutions at 284 nm. However, as other compounds in honey (predominantly phenolics) may also absorb at this wavelength. Therefore, a correction is made by the addition of sodium bisulphite solution to the honey and measuring its absorbance at 330 nm. The difference between the two absorbance values corrects the effects of interfering compounds.

2.3.3. pH and free acidity content

pH was measured according to [11] where the pH of a solution of 10 g of honey in 75 mL of water was determined by employing a magnetic stirrer and a (pHS-550) pH meter.
Total free acidity was determined for the same solution by titration against 0.1 M NaOH solution to pH 8.30 taking into account that the process would not take longer than 2 min to get to a final steady reading of pH.

2.3.4. Total sugars

Reducing and total sugar content was determined following Lane-Eynon method [11], where sucrose and reducing sugars (represented by glucose and fructose) was determined titrimetrically using a modified Fehling method that measures reducing sugar content before and after acid hydrolysis of honey. Modified Fehling solution was titrated at boiling point against honey solution (0.1%) using methylene blue as an indicator. Honey samples were treated with HCl at 65^{o}C in order to convert sucrose to glucose and fructose. The excess acid was neutralised with NaOH and the resulting solution was used for titration as before. The increase in reducing sugar values after hydrolyses reflects the amount of sucrose present in the samples.

2.3.5. Diastase activity

Diastase activity was determined by applying modified Schade's method [12]. Ten millilitres of (20%) honey solution containing NaCl and acetate buffer was brought to equilibrium with equal volume of 2% aqueous starch solution in a 40^{o}C water bath for 15 min. After which, 5 mL of the starch solution was added to the honey solution, mixed well and kept in the same temperature water bath. Aliquots of 0.5 mL were periodically removed from this solution and added to 5 mL iodine solution and 20 mL of distilled water (exact

water volume was determined from calibration of starch solution). The resulting solution was mixed well and its absorbance was recorded at 660 nm using water as blank. Subsequent aliquots were removed at different time intervals covering a range of absorbance values from 0.770 to below 0.200. The value of tx (time at absorbance value 0.235) was determined applying the linear regression equation for the data plot of time versus absorbance. Diastase number was calculated as 300/tx.

2.3.6. Folin-Ciocalteu test for phenols

High quality samples from both groups were selected. This included samples with a combination of HMF content less than 40 mg kg^{-1}, levels of diastase value >10 and electrical conductivity above 300 µS cm^{-1} for the determination of TPC and antioxidant activity measurements. TPC analysis was performed according to the method modified by Piljac-Žegarac *et al* (2009) [13].

2.3.7. DPPH Radical scavenging assay

Antiradical activity of honey samples was determined according to the method described by Molyneux [14].

2.4. Antibacterial assay

2.4.1. Specimen collection and samples sources

Five bacterial isolates of each of *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* were attained from clinical specimens (blood, sputum, urine and pus from wounds), submitted to be tested for bacteriology tests from patients admitted to the hospitals in Erbil city, Iraq. The specimens were taken and primarily inoculated onto MacConkey and blood agar medium (Oxoid, UK) and incubated at 37°C for 24 hr. From the single colonies, each bacterial isolate was identified conventionally by culturing in selective media (Salmonella Shigella, Mannitol Salt agar medium (Oxoid, UK) for both *S. typhi* and *S. aureus* respectively, and Cetrimide agar (acumedia, Neogen, USA) for *P.aeruginosa,* and by various biochemical and conventional diagnostic tests as described previously by Tille (2017) [15]. Bacterial isolates were further identified by Vitek II automated system (bioMérieuxMarcyl'´Etoile, France) (Vitek Systems Version: 06.01). Furthermore, the bacterial isolates were tested for their susceptibility to a panel of antimicrobials (amikacin, cefepime, cefotaxime, cefoxitin, ceftazidime, cefuroxime, chloramphenicol, ciprofloxacin, doxycycline, gentamycin, imipenem, meropenem, netilmicin, norfloxacin, tobramycin, trimethoprim and trimethoprim-sulfamethoxazole) by Vitek II automated system and disc diffusion method.

2.4.2. Honey samples for antibacterial activity

Six crude, unprocessed and undiluted samples from both KRI and AG honey were selected randomly to test their antibacterial activity against the isolated MDR bacteria.

2.4.3. Purity test

The purity of the honey samples was tested by inoculating a loopful of undiluted honey onto MacConkey, blood agar and Sabouraud dextrose agar media (Oxoid, UK). After incubation at 37^{o} C for 24 hr, the pure honey was selected for antibacterial assay.

2.4.4. Determination of minimum inhibitory concentrations (MIC)

Agar dilution method was used to determine the MIC for both KRI and AG honey samples against the identified MDR bacterial isolates [16]. Briefly, a volume of liquid honey was added to melted Mueller Hinton agar (MHA, Oxoid, UK) at 56^{o}C giving the final concentrations of 2.5, 5, 10, 15, 20, 25 and 30%. The plates were then inoculated by streaking

overnight culture of each bacterial isolate stationary phase equilibrated to OD550=0.5) on MH-honey agar media. Then the plates were incubated aerobically at 37°C for 24 hr. The lowest concentration with no evident growth was determined as MIC. Plates of MHA without honey inoculated with bacterial isolates served as control. Three biological replicates were considered on distinct occasions.

2.5. Statistical analysis

The assay results were analysed using GraphPad Prism 8.0 software. Un-paired t-test for significance was performed to compare between KRI and AG honey. One-way analysis of variance (ANOVA) method was used for multiple comparisons.

3. Results and Discussion

Honey samples were evaluated and compared on the basis of Codex Alimentarius International Food Standards - Standard for honey (CXS 12-1981) in its 2019-amended version [11], European Honey Directive [17] regulations and taking into account points of differences between the two standards [18], [19].

When honey samples are heated, the most prominent deterioration product is HMF. This compound is formed through Maillard reactions when hexose sugars are dehydrated by a strong acid like H_2SO_4 in experimental conditions. Presence of this compound in honey samples has been taken as an indicator for exposure to high temperature either due to initial processing, aging or inappropriate storage conditions. The studied honey samples showed HMF levels in the range 17.2±6.39 for KRI and (24.26±7.89) for AG samples. Both value ranges are considered normal and acceptable. A number of samples within each group showed exceptionally high levels of HMF which might be due to heat exposure. Also, comparison between the two sample groups showed that HMF values from KRI were lower than AG samples. However, these differences were non-significant (Table 1and 2, Figure 1-A). HMF levels showed negative correlation with diastase activity. However, it has been suggested that this negative correlation may not be considered a reliable indicator of honey quality, this is due to that diastase activity can be more time-dependent rather than temperature-sensitive. Furthermore, heat and storage can affect HMF and diastase differently even for honey from similar origin [20].

Table 1: Biochemical parameters for Kurdistan Region-Iraq honey samples

Sample Mumber	HMF	pH	Total Acidity	Water %	%Total Sugar	%Reducing Sugar	% Sucrose	Electrical Conductance	Diastase Number	TPC mg.100g^{-1} (GAE)	Antioxidant Activity %
1	8.5	3.78	30	14.6	80	80.0	0.0	378	50.0	141.4	45.4
2	3.4	3.80	28	15.4	78	78.0	0.3	443	46.0	105.1	44.9
3	21.3	3.78	39	21.3	71	69.4	1.9	416	29.0	97.7	55.1
4	4.8	7.05	10	13.9	70	66.7	3.8	1063	23.0	128.9	59.0
5	1.5	3.80	25	15.5	79	78.5	0.5	370	33.0	102.1	29.0
6	62.0	3.95	23	16.0	77	77.5	0.0	450	20.0	ND	ND
7	37.1	4.03	23	16.0	81	74.6	7.9	190	10.0	87.3	54.2
8	9.7	3.62	10	14.0	72	69.4	3.0	530	4.5	ND	ND
9	3.7	3.90	22	14.5	80	78.0	2.0	321	35.0	ND	ND
10	91.9	3.96	24	15.1	83	75.2	8.2	486	28.6	ND	ND
11	3.1	3.97	22	14.0	83	83.0	0.0	351	53.0	142.5	55.5
12	6.4	3.77	28	13.0	84	84.6	0.0	307	13.6	ND	ND

13	5.3	3.84	24	14.0	84	79.0	0.6	435	35.9	90.0	24.3
14	2.7	3.90	25	14.0	84	82.3	2.3	357	40.0	70.1	45.9
15	9.3	3.73	31	15.2	83	79.9	3.9	360	59.0	67.8	13.9
16	4.0	4.35	35	15.0	79	78.0	1.1	550	31.5	77.0	34.0
Accepted value	≤ 80 mg.kg^{-1}	3.5 – 6.1	< 50 mmoles.kg^{-1}	≤ 23%	<83%	<83%	<5%	<800 µS.cm^{-1}	>80 U		
Mean± SE	**17.2± 6.4**	**4.08 ±0.15**	**24.97 ±1.89**	**13.34 ±1.39**	**79.25±1.68**	**77.0±1.3**	**2.0± 0.76**	**438±47**	**32.01 ±3.87**	**101 ±8.04**	**51.6 ±3.6**

* ND: not determined
(GAE): gallic acid equivalent

Table 2: Biochemical parameters for Arab Gulf honey samples

Sample Number	HMF	pH	Total Acidity	Water %	%Total Sugar	%Reducing Sugar	% Sucrose	Electrical Conductance	Diastase Number	TPC mg.100g^{-1} (GAE)	Antioxidant Activity %
1	97.3	3.72	36	15.6	78	75.5	2.6	417	4.1	ND	ND
2	0.0	5.23	8	14.6	79	74.7	4.4	750	14.2	49.0	24.4
3	56.7	3.99	18	15.4	80	74.7	5.3	389	18.1	28.3	5.9
4	72.9	4.18	18	18.0	79	76.4	2.7	331	12.2	ND	ND
5	0.0	5.32	16	15.6	76	76.4	0.0	784	23.3	63.2	22.9
6	51.8	4.46	22	15.0	78	78.1	0.0	499	7.0	ND	ND
7	0.0	5.30	11	14.8	80	76.4	3.6	777	17.3	59.1	25.1
8	9.3	5.55	11	14.8	75	74.7	0.0	948	15.4	54.0	20.1
9	13.3	4.98	20	14.8	80	74.7	5.3	394	9.1	47.7	8.0
10	0.2	4.00	70	17.4	82	79.1	2.9	372	0.2	ND	ND
11	53.6	5.40	13	13.4	80	78.1	1.9	770	4.9	ND	ND
12	0.0	5.08	15	15.8	75	73.9	0.8	750	12.1	57.8	14.5
13	17.9	4.36	16	14.6	76	75.5	0.9	728	15.9	44.6	11.7
14	15.1	4.87	20	14.8	75	74.7	0.0	758	3.9	ND	ND
15	0.0	5.19	12	16.2	75	74.7	0.0	798	13.9	60.7	19.7
16	0.0	5.10	11	14.0	75	74.7	0.0	752	5.6	ND	ND
Accepted value	≤ 80 mg.kg^{-1}	3.5 – 6.1	< 50 mmoles.kg^{-1}	≤ 23%	<83%	<83%	<5%	<800 µS.cm^{-1}	>80 U		
Mean ±SE	**24.26± 7.89**	**4.79± 0.20**	**19.81± 3.72**	**15.3± 0.29**	**77.69± 0.60**	**76.0± 0.38**	**1.9± 0.5**	**639±50**	**11.08± 1.59**	**41.9± 4.44**	**16.9±2.39**

* ND: not determined
(GAE): gallic acid equivalent

Gluconic acid is the main acidic compound in honey with concentration around 0.5%. Despite their small quantities, acidic compounds are responsible for a number of important chemical and physical characteristics in honey [21]. Both sample groups showed pH and total acidity values in the accepted range with KRI honey having 4.08±0.15 and 24.97±1.89, and AG samples 4.79±0.20 and 19.81±3.72 for pH and total acidity values respectively (Table 1, 2 and Figure 1-B and C). KRI honey showed comparatively lower pH values and the

differences were significant at $P \leq 0.001$, while relatively higher acidity was found to be non-significant.

Water content of honey affects its stability in a manner similar to the effects of pH. Lowering water content is found to improve quality of honey [22]. This means higher stability, prevention of microbial growth, storage over longer time and generally higher chemical and physical qualities [23]. This is why attempts are ongoing by beekeepers and researchers alike for developing techniques for water evaporation and reduction of honey moisture [24]. Water content of KRI and AG samples in this research were 13.34±1.39 and 15.3±0.29 respectively (Figure 1-D). Both values were at desirable level and in agreement with permitted values.

Sugar analysis results were within acceptable limits for both groups and showed no significant difference between the two sample groups. KRI and AG samples contained total sugars of 79.25±1.68% and 77.69±0.60% of which reducing sugars accounted for 77.0±1.3% and 76.0±0.38%, and sucrose for 2.0±0.76% and 1.9±0.5% of total honey weight for both groups respectively. This rather low content of sucrose is the cause for the low glycemic index of honey [25]. However, this characteristic often shows some variation depending on the honey type. Both sample groups in this study showed satisfying quality in this regard. Some samples from both groups showed higher values of sucrose content as can be seen from the variability from mean value (Figure 1G) which can indicate inadequate feeding practices and/or premature harvest of honey in which case sucrose has not been completely hydrolysed into glucose and fructose [26].

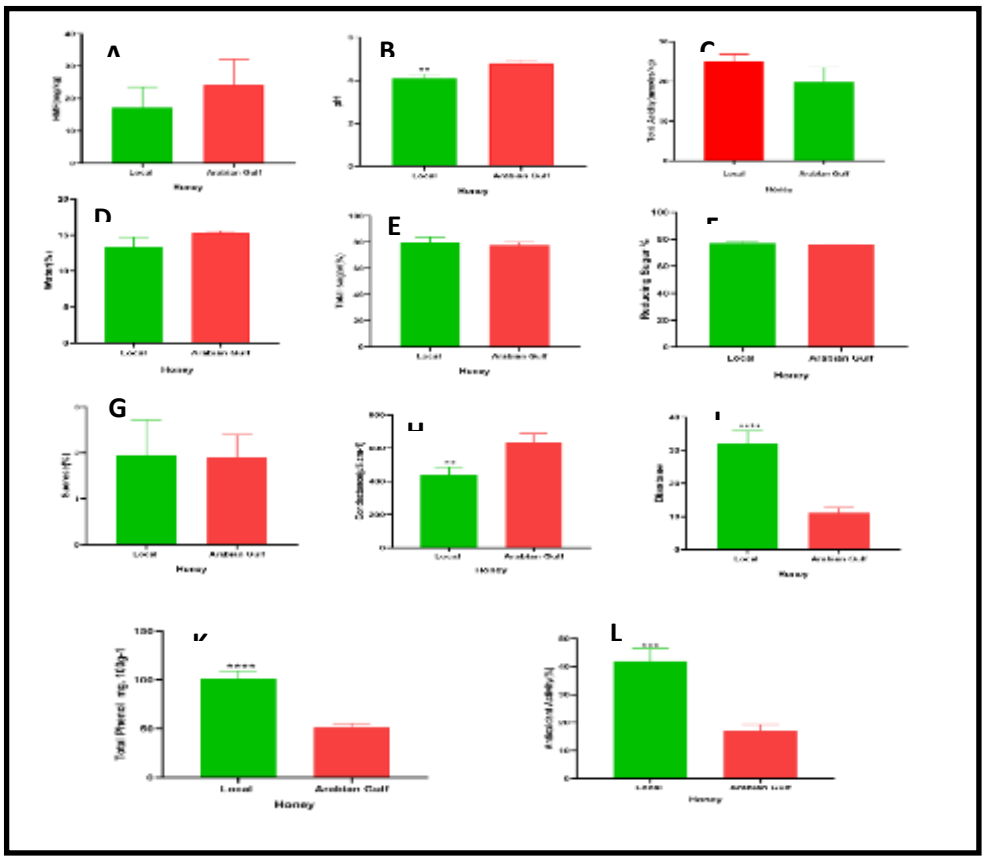

Figure 1: Comparison of biochemical parameters between KRI and AG honeys. A: HMF, B: pH, C: Total Acidity, D: Water%, E: Total sugars, F: Reducing sugars, G: Sucrose%, H:Conductance, I: Diastase, K: TPC, L: Antioxidant activity. **; significance at $P \leq 0.01$, ***; p<0.001, ****; $P \leq 0.0001$.

Electrical conductivity values of different honey samples from KRI and AG were 438±47 and 639±50 respectively. These results were significantly different ($P \leq 0.01$) but were both within normal range set by codex standard at less than 0.8 mS/cm for a 20% aqueous solution of honey and in agreement with standards.

Diastase number was found to be significantly different between both investigated honey groups ($P \leq 0.0001$), with KRI honey showing pronouncedly higher levels. This enzyme sources from the honeybee's saliva secretions and its content being sensitive to high temperatures and improper processing that may cause its degradation [27]. Other sources of variability include the bee species as explained by Vit and Pulcini (1996) [28]. Diastase number and HMF are used together as an indication for honey freshness and authenticity [29]. Accordingly, and taking into account that both sample groups are produced mainly by *Apis mellifera* bee species, KRI honey will have more desirable quality when considering a combination of lower HMF and higher diastase number in comparison to AG honey.

Variation in honey quality attributes between the KRI and AG sample groups can source from differences in environmental factors and geographical location of the hive which can affect the specific climate characteristics of the region. As the nature of Iraqi Kurdistan region with its mountainous range where beehives are mostly kept can be different both in elevation and flora type from the geographical location of beehives in Arab Gulf countries [30]. These variables have been shown to cause variation in biochemical parameters of honey such as acidity [21], water content [31], electrical conductivity [32] and diastase number [33]. Additionally, processing and storage conditions, and degree of maturity of honey also contribute to the variation [31].

contained (101±8.04) mg.100g^{-1} gallic acid equivalent of phenolic compounds which was significantly higher at $P \leq 0.0001$ from AG samples at (51.6±3.6) mg.100g^{-1}. Elevated TPC are often perceived as darker colour in honey and associated with high quality, better nutritional value and more beneficial health effects [1]. Phenolic compounds impart a significant biological activity to honey due to their antioxidant power that can counteract the damage caused by free radicals in the body [34], [35]. Antioxidant potential of honey is therefore directly proportional to the amount of polyphenolic compounds present in it. This has been observed in the present study as antioxidant activity was found to be 41.9±4.44 % for KRI versus 16.9±2.39 % for AG samples with $P \leq 0.001$ (Figure 1L).

The fact that the infectious diseases caused by MDR microorganisms lead to the death of sixty million individuals every year led to the need for alternative approaches to fight these MDR microbes [36]. One strategy is to use natural remedies like honey and medicinal plants. The efficacy of different types of honey as antibacterial was evaluated on many MDR pathogens, five clinical bacterial isolates (*A. baumannii*, *E. coli*, *P. aeruginosa*, *S. typhi*, and *S. aureus*) from different specimens (Figure 2). According to the results of susceptibility test, all isolates were resistant to all antimicrobials used, consequently the isolates were considered as MDR bacteria. To test the efficacy of honey on MDR isolates, MICs were determined by agar dilution method. The local honey samples exhibited significant inhibition of bacterial growth with mean of MICs between 11±1.00 - 23±1.23. Whereas AG honey required higher concentrations to inhibit the bacterial growth with 19±1.00 - 25±0.00. Furthermore, the obtained data showed that *S. typhi* isolates were more sensitive to local honey samples with mean MIC of 14.2±2.01 (Table 3 and Figure 3). When compared, KRI honey samples recorded high significant differences with lower MICs of approximately 5% than AG honey (Figure 3C). The findings demonstrated that the growth of all these pathogens affected by all

honey types at different concentrations. Similar findings were recorded by Lusby (2005) [37] and Al-Hasani (2018) [38]. Various significant factors contribute to honey antimicrobial efficiency, including low pH, low H_2O content, osmolarity, phenolic acid and flavonoids levels [8].

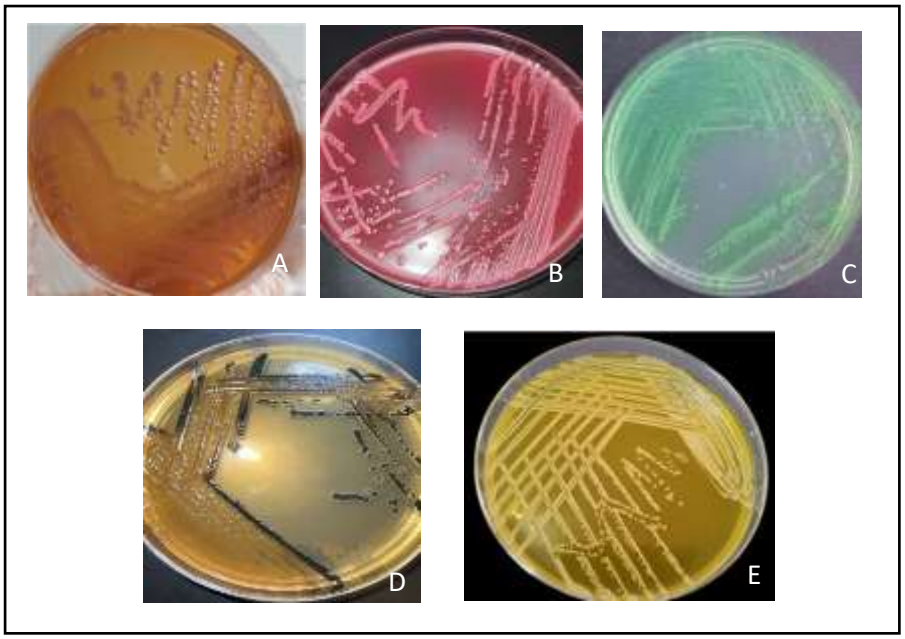

Figure 2: Colony morphology of the studied bacterial isolates on their selective media; A: *Acinetobacter baumannii*on MacConkey Agar*;* B: *Escherichia coli* on MacConkey Agar, C: *Pseudomonas aeruginosa on* Cetrimide Agar, D: *Salmonella typhi* on Salmonella Shigella Agar, E: *Staphylococcus aureus* on Mannitol Salt agar.

Table 3: MIC of Kurdistan region, Iraq (KRI) and Arab Gulf (AG) honey against bacterial pathogens

		MIC (v/v%) for different test pathogen					Mean±SE
Sample Group	**Sample Number**	***A. baumannii***	***E. coli***	***P. aeruginosa***	***S. typhi***	***S. aureus* (MRSA)**	
KRI Honey	**1**	20	20	20	20	20	20±0.00
	2	10	15	15	10	15	13±1.23
	3	10	15	10	10	10	11±1.00
	5	25	20	25	20	25	23±1.23
	6	20	20	20	10	20	18±2.00
	11	15	15	15	15	15	15±0.00
	Mean±SE	**16.7±2.47**	**17.5±1.12**	**17.5±2.14**	**14.2±2.01**	**17.5±2.14**	
AG Honey	**2**	20	20	20	15	20	19 ±1.00
	8	20	20	20	20	25	21±1.00
	9	25	25	25	25	25	25±0.00
	12	20	20	20	20	20	20±0.00
	13	20	20	20	20	20	20±0.00
	16	20	20	20	20	20	20±0.00
	Mean±SE	**20.8±0.84**	**20.8±0.84**	**20.8±0.84**	**20.0±1.29**	**21.7±1.05**	

Data denote mean values of three independent experiments.

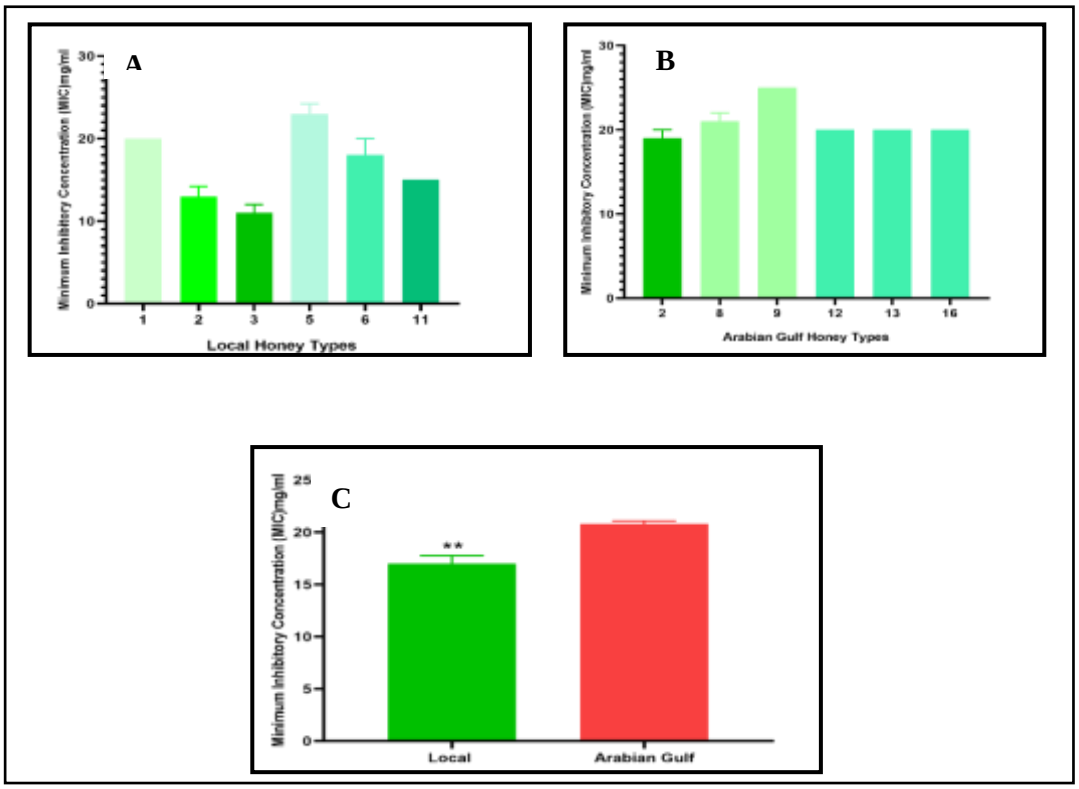

Figure 3: MIC of (A) Local and (B) AG honeys. (C) Comparison of MIC of local KRI and AG honeys. **; P<0.01. Data denote mean values of three independent experiments.

The response of bacterial isolates to honey sample varied for both pathogen and honey types, but in general local samples showed lower inhibitory concentrations. *Salmonella typhi* showed inhibition by lower honey concentration (14.2±2.01) when compared with other bacterial isolates. Statistically there was a significant difference between the antibacterial activity of the two honey types with the Kurdish honey being more effective than AG honey. This can be resulted by the different physical and biochemical factors presented in our results such as water %, pH and acidity, and TPC and antioxidant activity. The water % in KRI honey was lower than that of AG honeys.

Water is essential for all living organisms and it exists in the form of bound or free molecules. In honey, water content is low enough to prevent the bacterial growth [39]. Another significant difference is in pH. Lower pH of local honey could have an important role in giving rise to the antimicrobial effect in the study. Most microorganisms prefer growth at neutral pH, varying between 6.5 to 7.5. Honey pH (3.2-4.5) is a very marked feature of its inhibitory efficacy. Furthermore, the type of flowers from which honey is sourced (which is mostly related to their geographical origin) is found to be key factor in determining antibacterial and antioxidant activity of honey [40]. This further concludes the fact that high TPC in local honey reported in our data may have a vital role in its significantly higher antibacterial effectiveness.

4. Conclusion

Honey samples both from KRI and AG showed physical and chemical quality attributes within the normal and acceptable values in general. Occasionally, some of the samples in both groups showed undesirable traits which can be due to improper bee keeping practices as well

as packaging and storage. For the first time, the study revealed significantly high quality of Iraqi honey from Kurdistan region. This high quality is based on the high records of diastase enzyme, total phenolic content and antioxidant activity. This in turn was seen as high antibacterial activities. These desirable characteristics should be preserved and enhanced by applying more regulations by the government and providing support for the local beekeepers

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Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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