Original Article

Biological Activities of Natural Honey Produced In Basrah Governorate

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Abstract

Honey is a unique food product that contains biologically active compounds derived from Bees and plants. These bioactive compounds can be linked to antimicrobial activity has the ability to destroy or inhibit the growth of certain pathogenic microorganisms. Three kinds of local honey were collected in Basrah governorate: *Ziziphus* (Sidr) honey (S), *Medicago* (Barsim) honey (B), and *Eucalyptus* (Calbtose honey) (C). Collected from honey shops in the province. The antibacterial potential of each honey was evaluated using 12 pathogenic strains, Gram positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia, Staphylococcus* spp.), Gram negative (*Escherichia coli, Proteus vulgaris, Pseudonetum aeruginosa, Acinetobacter, and Pseudomonasus spp*.), and fungi (*Aspergillus niger*, *Candida albicans* and *Candida glabrata*). The results of the antibacterial activity against bacteria and fungi showed all honey types were significantly effective against pathogenic isolates. The highest values of the inhibition zone were about 50 mm by *Ziziphus* (Sidr) honey (S). The *Eucalyptus* (Calbtose honey) (C) showed lower values compared to the other types. The biological activity against fungi (Antifungal) showed the different types of honey had less effect than the bacteria. The results of antioxidants showed that all types of honey showed different actions, the highest by Ziziphus (Sidr) honey (S) (60%), followed by Medicago (Barsim) honey (B) (55%) and the lowest was (53%) by Calbtose honey (C). The bioactivity of honey on the biofilm was showed the highest values of Biofilm inhibition level was formed by ***Staphylococcus aureus*** at concentrations of 25 % of Barsim (B) compared to the other types, on the other side the maximum inhibition of biofilm was formed by *Pseudomonas aeruginosa* at concentrations 25 % was for Barsim (B).

Keywords: Honey, Antioxidants, Biofilm, Biological Activity.

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INTRODUCTION

Since antique times, almost 5500 years ago, honey has been consumed by humans (Adebolu, 2005). Most ancient people, including the Greeks, Chinese, Egyptians, Romans, Mayans, and Babylonians, ate honey both for dietary purposes and for its medicinal properties (Andrzej et al., 2018).

(Samarghandian et al., 2017) shown that honey can have a medicinal effect due to its anti-bacterial, anti-inflammatory, apoptotic, and antioxidant properties, this research could give practitioners substantial evidence to support the use of honey in the medical field. Compounds that have antioxidant and antibacterial properties are found in honey, particularly with gram-positive microorganisms, *staphylococcus*, and *Bacillus*, it may have a relevant role in the study products to mitigate the symptoms of infections with bacteria and contribute to healthier meals (Bueno-Costa et al., 2016). (Velásquez et al., 2020) reported that *Staphylococcus aureus*, *S. pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* were inhibited by all phenolic compounds extracted from honey samples.

Carbohydrates, mainly fructose and glucose, are components of honey, with remaining sugars including maltose, sucrose, and other complex carbohydrates. Honey also includes small yet important quantities of biologically active phenolic compounds, minerals, vitamins, amino acids, proteins, enzymes, organic acids, and other phytochemicals, in addition to carbohydrates (Bueno-Costa et al., 2016); (Kasprzyk et al., 2018). The chemical composition depends on the botanical and geographical origin of the source of honey, as well as the environmental conditions (Bogdanov et al., 2008).

Biofilms are sessile bacterial populations trapped in an extracellular polymeric matrix. Compared to cells living in a planktonic setting, bacterial cells trapped in biofilms are fundamentally recalcitrant to antimicrobials and are extremely hard to remove once established (Mathur et al., 2018). Biofilms have been documented to lead to physical, chemical, and antimicrobial defense (Donlan, 2002). The persistence of biofilms on medical instruments and surgical facilities in clinical environments enables some pathogens to infect patients easily (Kostakioti et al., 2013). Moreover, 70% of pathogenic bacteria have been shown to be immune to antibiotics currently in use (Katz et al., 2006). In order to tackle drug-resistant pathogens, synthesis and development of novel antimicrobials are therefore needed (Stagos et al., 2018) considering honey's multiple contributions to human health the present study aimed to compare three types of local honey by evaluating their chemical composition, antibacterial and antioxidant activity.

MATERIAL AND METHOD

Sample collection

Three types of honey *Ziziphus* Sidr honey (S), *Medicago* Barsim honey (B), and *Eucalyptus* Calbtose honey (C) were purchased from Basra city markets in a 100-gram package, which is available as a licensed commercial medical device (Basra, Iraq). It is 100% pure honey derived from the plant *Ziziphus spina-christi, Medicago sativa,* and *Eucalyptus camaldulensis* in Basra, Iraq**.**

Physiochemical properties of honey

To ascertain the purity of the honey produced, the pH of the honey and the conductivity were digitally measured digitally at 25 °C using an electric pH meter. These characteristics are considered among the main characteristics for consumers and specialists, which reflect the quality and purity of honey, as well as its basic components.

Fourier transform infrared spectroscopy

The honey samples were subjected to Fourier transform infrared spectroscopy (FTIR) and recorded in KBr pellets, in FTIR Spectrophotometer at the range (450-3900) cm^{-1}. for the determination of functional groups. This was achieved at the department of chemistry/college of the Education / University of Basrah.

Compounds profile by GC-Mass spectroscopy

Compounds in three types of honey were identified based on their mass spectral data by using Gas chromatography- Mass spectroscopy at Nahran Omer laboratories, analysis was done in a Shimadzu Qp 2010 quadrupole GC-Ms, equipped with a capillary inlet at (30m x 0.25 mmID; 0.25 in film thickness) the samples were injected in the following conditions: injector temperature 300℃; carrier gas, helium; pressure,11.962 psi.

Microorganisms

Different pathogenic isolates were provided by the Department of Biology, College of Science, University of Basrah, as followed: Gram negative bacteria *Acientobacter baumanii*, *Acientobacter baylyi*, *Escherichia coli***,** *Proteus mirabilis***,** *Pseudomonas aeruginosa, Proteus vulgaris*), Gram positive bacteria (*Staphylococcus aureus***,** *Staphylococcus hominies***,** *Staphylococcus pseudintermedius, Streptococcus pneumonia***,** *Streptococcus pyogenes*, and fungi (*Aspergillus niger, Candida albicans* and *Candida glabrata*). On sterile nutrient agar plates and nutrient broth tubing, bacterial strains were regularly preserved and cultured, and fungi have been grown on Potato Dextrose Agar Petri dishes.

Antimicrobial activity

The antimicrobial assay was performed on Mulleur Hinton agar medium by agar well diffusion method. 0.1 ml of inoculum with a concentration of 0.5 MacFarlend's stander was applied to the surface of the sterile agar plates and spread uniformly. After drying, 7mm diameter wells were made by using a sterilized core borer.100 ul of crude honey was added. The plates were incubated for 24 hours at 37°C (Perez, 1990). Measured and registered the non-growth halo diameter (inhibition zone) in diameter. For fungi, the same method was followed by the use of a Potato Dextrose agar medium (PDA) with a concentration of fungal spore suspension of $1x10^6$ cell /mL.

Antioxidant assay

The free radical scavenging potential of DPPH for honey was assessed, 1 ml of honey was blended with 1 mL of freshly prepared methanol DPPH (0.004%) thoroughly vortexed, incubated for 30 minutes at room temperature in the dark, methanol with DPPH was used as a blank and ascorbic acid as control. Using a UV-Vis spectrophotometer, absorbance was registered at 517nm (Mohanty et al., 2014). The operation of free radical scavenging was expressed as the percentage of inhibition calculated using the following equation:

DPPH (%) Radical scavenging = Control OD- Sample OD/ Control OD X 100

Antibiofilm assay

Minimum inhibitory concentrations (MIC) for three types of honey were established using a range of concentrations of (12.5-100% (w/v), in a total volume of 50µl nutrient broth in a 96 well microtiter plate.

Minimum biofilm eliminating concentration.

All strains were cultured aerobically at 37°C in 5 ml nutrient broth (NB) (Oxoid, Cambridge, UK) for 24hrs. After the cultured microorganism reached mid-exponential phase, harvested and adjusted the Optical density $_{600} = 0.05$. Biofilms were established in 96 well microtitre plates in 200 µl NB containing a five range of concentrations of three types of honey (12.5, 25, 50,75, and100% (w/v), by inoculating each well with 50 µl of equilibrated cells. Plates were incubated at 37°C for 48h (Campeau & Patel, 2014). All experiments were carried out in triplicate.

Crystal violet assay (Microtiter plate assay)

After 48hr. incubation the pates were used to determine the quantification of *Staphylococcus aureus*, and *Pseudomonas aeruginosa* biofilm growth, unattached planktonic cells were gently aspirated and discarded, biofilms were fixed with 200µl 99% methanol for 15min. Biofilms cells were washed twice with PBS and then all wells were stained with 200µl crystal violet (1% w/v) for 20min. After a subsequent two washes with PBS, cell-bound crystal violet was re-solubilized with 200µl of 95% ethanol for 30min. 100µl of ethanol was transferred to a new plate from each well to measure the absorbance at A_{490}. (Haney et al., 2015).

Statistical analysis

The analysis of variance (ANOVA) LSD (least significant difference 0.05 was applied) and principal component analysis (PCA) was used to achieve the suitability of the analyzed parameters for botanical authentication of honey. ANOVA was carried out using Statgraphics.

Results and Discussion

Honey properties

Natural honey includes numerous substances in the contents of compounds according to floral references, giving honey different properties. The physical and chemical properties showed the S honey had a high moisture content of 19.2% then C honey was 7.2, while the lowest moisture in B honey was 3.2%, pH value was (3.76-3.91), the color showed light amber to dark amber, specific gravity g/ml at 25°C of (S, C and B) honey were 1.669, 1.559 and 1.552 respectively. It is clear from reading the characteristics listed in the table that there is a convergence in the value of the acid exponent, electrical conductivity, and moisture content with a difference in morphological characteristics. The vegetation and climatic conditions that can affect the different properties of honey (James et al., 2009; Cantarelli et al., 2008; Ebenezer & Olugbenga, 2010).

Property	**S honey**	**C honey**	**B honey**
pH	3.91 ± 0.02	3.95 ± 0.02	3.76 ± 0.02
EC	178.7 ± 0.37	175.8 ± 0.38	186.8 ± 0.35
Color	Dark amber	Amber	Light amber
Total moisture %	19.2 ± 0.8	7.2 ± 0.7	3.2 ± 0.6
Specific gravity g/ml	1.669±0.07	1.559±0.08	1.552±0.1

Antibacterial assay

Antibacterial action against pathogenic bacteria by agar well diffusion assay revealed that *E. coli* and *p. mirabilis* were the most affected bacteria with inhibition zone (50) mm for all types of the honey groups, followed by *Acinetobacter spp*. and *S. hominis* (40) mm, *S. pyogenes* (30)mm, *P. vulgaris and S. pneumonia* (20). The antibacterial activity of the honey types is attributed to hydrogen peroxide and proteinaceous compounds (Stagos et al., 2018). Many studies have agreed that honey has antimicrobial activity that varies according to its composition, which in turn depends on the plant origin, geographical location, bee species and production seasons (Khan, 2014).

Table (1): Bacterial activity of three types of honey against pathogenic bacteria.

Cod	**Isolates**	**Inhibition zone (mm)**		
		Sider honey	**Sider honey**	**Calbtose honey**
1A	*P. vulgaris*	20	20	20
2B	*Acinetobacter spp*.	40	40	40
3C	*E.coli*	50	50	50
4D	*S. pyogenes*	30	30	0
6E	*S. pneumonia*	0	20	20
7F	*A. baylyi*	0	0	0
8G	*P. mirabilis*	50	50	50
9H	*S. aureus*	40	20	20
10J	*S. pseudintermedius*	40	30	20
12	*S. hominis*	40	40	40

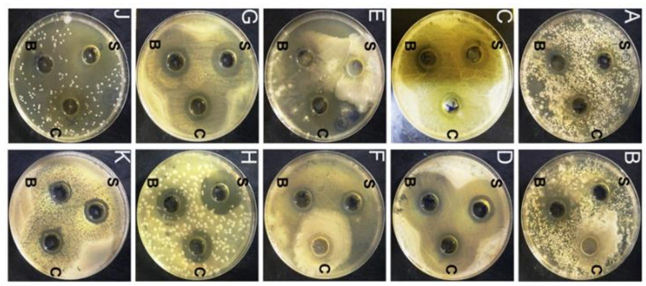


Figure 1. Antibacterial activity of Honeys against different pathogenic bacteria; S: Sider honey, C: Calbtose honey, B: Barsim honey, A: P. vulgaris, B: *Acinetobacter spp*., C: E coli, D: *S. pyogenes*, E: *S. pneumonia*, F: *A. baylyi*, G: P. mirabilis, H: S. aureus, J: S. pseudintermedius, K: S. hominis

Antifungal assay

The results of the biological activity of honey against fungi (Antifungal), the different types of honey had the lowest effect on bacteria. The three types showed activity against *Candida glabrata*, while no effect against the other fungi, *Aspergillus niger* and *C. albicans*, as shown in Table 2 and Figure 2.

Table (2): Antifungal activity of three types of honey against pathogenic bacteria.

Fungi	**Inhibition zone (mm)**		
	Sider honey	**Sider honey**	**Calbtose honey**
A. niger	0	0	0
C. albicans	0	0	0
C. gm mlabrata	20	20	20

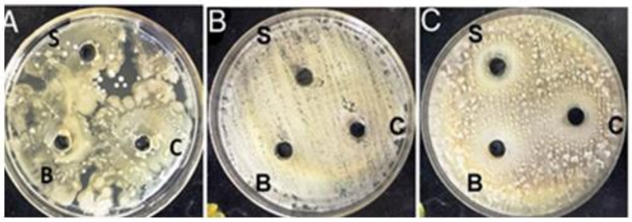


Figure (2): antifungal activity of honey against pathogenic isolates: A(A. niger), B(C. albican), C(C. glabrat), S: Sider honey, C: Calbtose honey, B: Barsim honey

Despite the lack of studies on the activity of honey as an antifungal, it was found in the current study that there is no inhibitory effect for all types of honey used in the study on two types of fungi ***A. niger*** and ***C. albican,*** while there was an equal inhibitor for three types on the fungus ***C. glabrat.*** DeMera and Angert, (2004) indicated that the ability of honey to inhibit the growth of yeasts varies according to the different geographic regions, and this proves the effect of the product of plant origin in affecting the antifungal activity. Khosravi et al., 2008, reported that honey has antifungal activity against Candida species such as ***C.dubliniensis, C. parapsilosis, C. kefyr, C. albicans, C. glabrata, and C. tropicalis.***

Antibiofilm Assay

The results of biofilm inhibition with different types of honey showed in figures (3). The highest inhibition at concentrations of 100 and 75 of Sider honey was (47.7 and 38.5%), respectively against *Staphylococcus aureus*, while the concentrations (50, 25, 12.5%) of Calbtose honey appeared high efficiency in inhibition of biofilms were 37.3, 37.4 and 33.6%. Barsim honey was shown to be less effective in inhibiting the biofilm formed by *Staphylococcus aureus*.

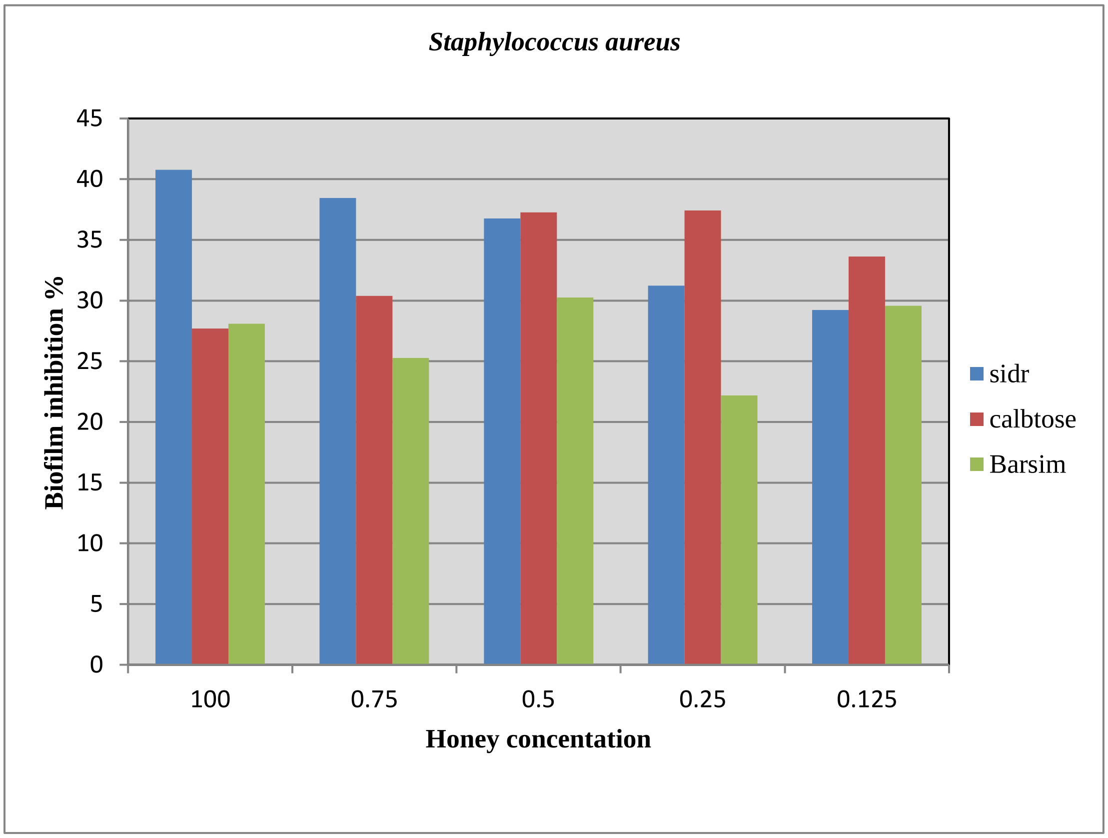


Figure 3: Biofilm inhibition % by different concentrations of various types of honey against Staphylococ\cus aureus

The results shown in Fig. 3 revealed the effect of biofilm inhibition of honey types at different concentrations. The maximum inhibition of biofilm was formed by ***Staphylococcus aureus*** at concentrations of 25, 75 and 100 % of Barsim honey was (**60.5,** 69.6, and 68.5%), while the concentrations (100, 75, 12.5%) of Calbtose honey demonstrated strong efficiency in biofilm inhibition at **37.3, 37.4** and 33.6 percent.

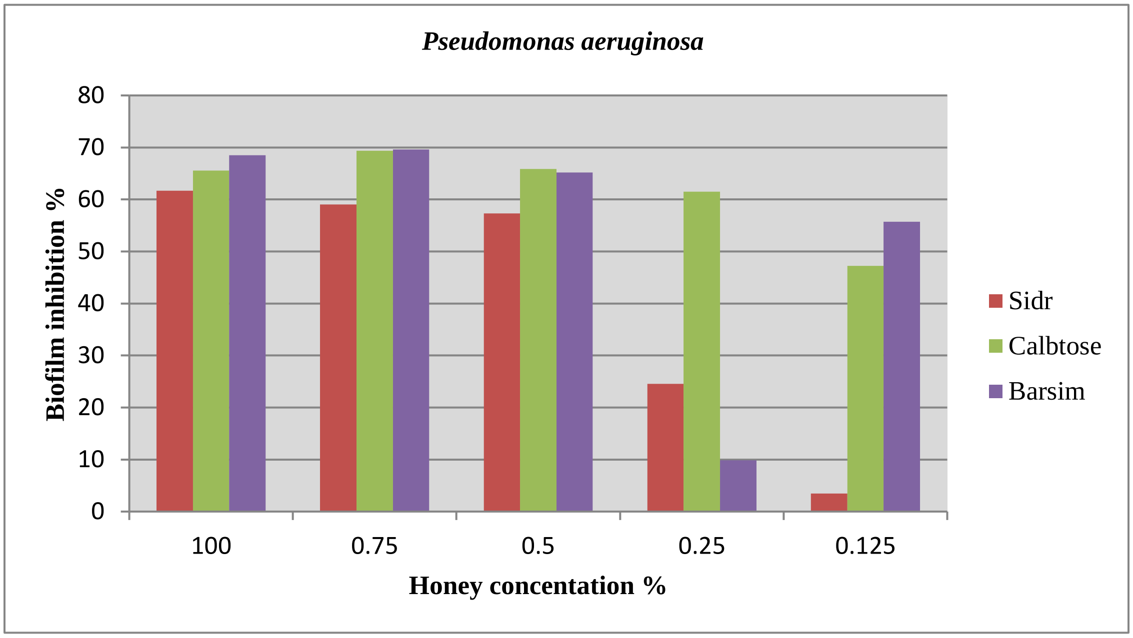


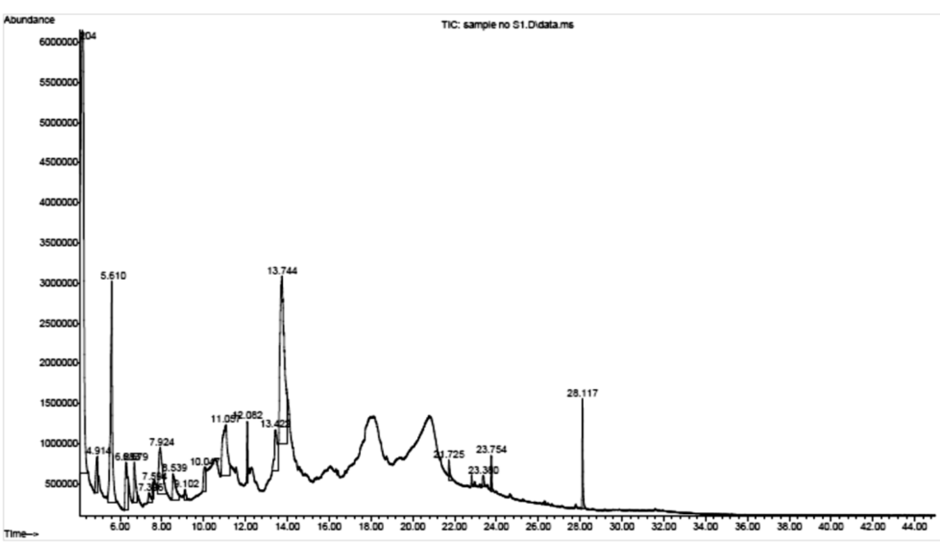
Figure 4: Biofilm inhibition % by different concentrations of various types of honey against Pseudomonas aeruginosa.

The results shown in Fig. 4 revealed the effect of biofilm inhibition of honey types at different concentrations. The maximum inhibition of biofilm was formed by *Pseudomonas aeruginosa* at concentrations of Sider honey was at 125 and 25% while the concentrations (25%) of Barsim honey demonstrated strong efficiency in biofilm inhibition, and the Calbtose Honey didn't show strong inhibition in all concentrations against *Pseudomonas aeruginosa*. Shirlaw et al., (2020) indicated that Manuka honey had an effect on reducing biofilm formation in**, *E. faecalis, P. aeruginosa, E. coli, and A. baumanii*** which agreed with what was presented by Maddocks, 2013 in previous studies. Shirlaw et al. (2020) suggested that the presence of phenolic compounds in honey was influencing the decrease in biofilm formation reported for Manuka honey by altering the structures of bacterial villi and flagella.

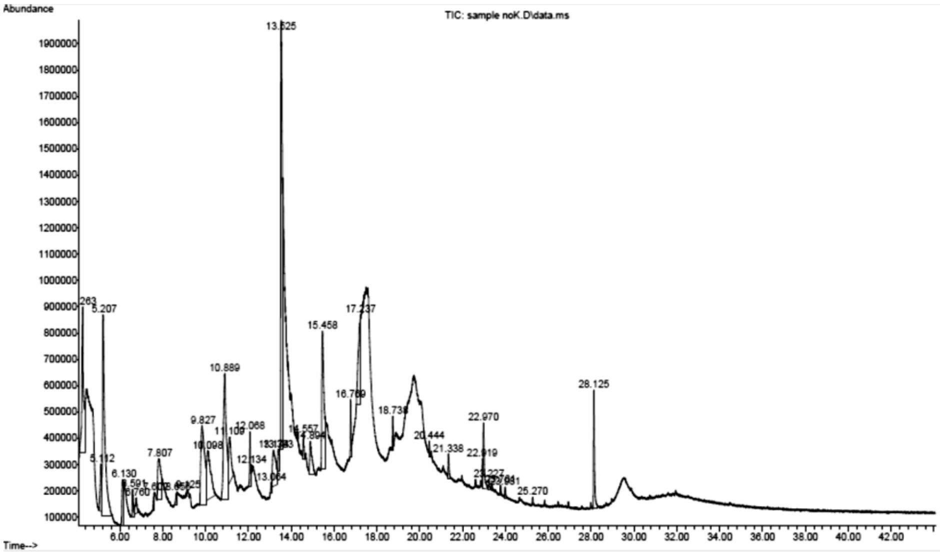
Mass Spectrometry (GC-MS) methods

The results of the GC mas spectrum graphic showed that the honey contained main compounds that are common to the three types of honey. **Figures 4,5 and 6** explain the rotation time and area peck of all compounds in different types of honey.

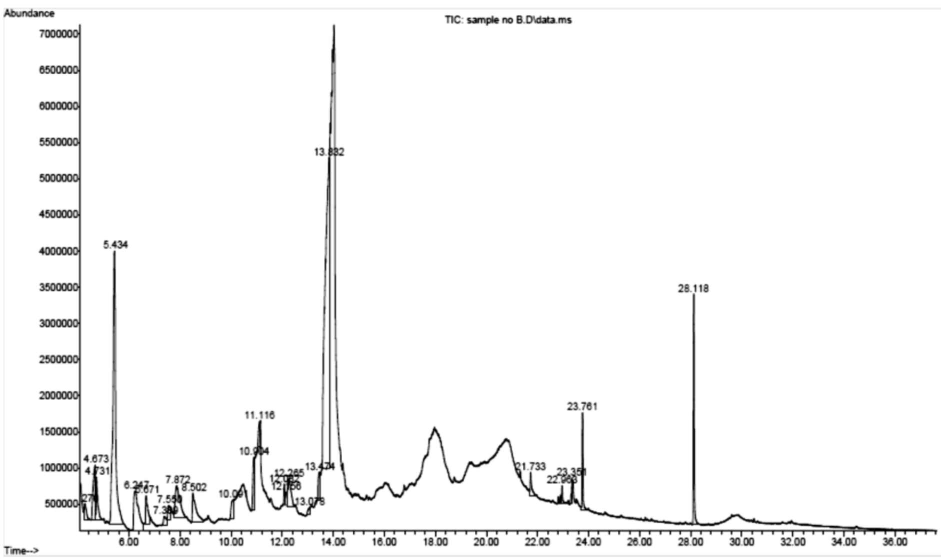
Details of the most important chemical compounds revealed by GC analysis are shown in the table 3.



Sidr



Calbtose



Barsim

Figure 4. GC mas spectrum of honey a: Sidr honey, b Calbtose honey, and c Barsim honey

Table 3. The results of the profile of honey by GC mas spectrum were showed there are some same compounds that present in all types of honey

S honey (19 compounds)				**C honey** (35 compounds)			**B honey** (24 compounds)		
No	RT	A%	Compound	RT	A%	Compound	RT	A%	Compound
1	4.2	35.7	Methanamine	4.26	6.5	2-Propanamine	4.67	3.23	l-Alanine
2	5.6	11.28	Trifluoropropene	5.23	11.9	Methanamine	5.43	21.6	Furfural
3	6.3	2.7	2-Furanmethanol	6.1	1.9	2-Furanmethanol	6.25	5.5	2-Furanmethanol
4	7.9	4.87	2(3H)-Furanone	7.8	3.5	2(3H)-Furanone	7.87	4.34	2(3H)-Furanone
5	8.5	2.3	Furancarboxaldehyde	8.65	0.4	3-Furanmethanol	8.5	3.14	2-Furancarboxaldehyde
6	-	-	-	9.827	7.99	Diaminohexane	-	-	-
7	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	10.9	2.68	Dimethylimidazole
9	-	-	-	10.098	4.78	Formaldehyde			
10	11.1	6.9	Furaneol	10.889	9.56	Furaneol	11.1	0.9	Furaneol
11	13.4	3.24	Catechol	11.109	2.8	propanamide	-	-	-
12				12.068	1.18	Hydrazine	12.1	0.5	5-Hydroxymethyl uracil
13				13.174	3.17	2(3H)-Furanone	13.5	1.5	Catechol
14	13.7	20.8	5-Hydroxymethyl Furfural	13.525	16.14	5-Hydroxymethylfurfural	13.8	35.9	5-Hydroxymethylfurfural - 4-Mercaptophenol
15	-	-	-	15.458	8.4	Isopropylimidazole-2-thione	23.4	0.8	Oleic Acid
16	-	-	-	17.237	5.62	Pentadecanoic acid	23.7	2.22	Adipic acid
17	28.1	2.28	Benzenedicarboxylic acid	28.125	2.92	Benzenedicarboxylic acid	28.1	5.25	Benzenedicarboxylic acid
18	-	-	-	-	-	-	12.26	2.5	4H-Pyran-4-one

The GC-MS analysis identified the following major chemical constituents namely Methanamine, Trifluoropropene, 2-Furanmethanol, 2(3H) Furanone, Furancarb-oxaldehyde, Furaneol, Catechol, 5-Hydroxymethyl Furfural, Benzenedicarboxylic acid. These compounds are known to have some biological activity such as antibacterial, antifungal, antioxidant (Awasum et al., 2015).

REFERENCES

Adebolu TT. Effect of natural honey on local isolates of diarrhea causing bacteria in Southwestern Nigeria. Afr J Biotechnol., 2005; 4: 1172–4.

Andrzej K. Kuropatnicki, Małgorzata Kłósek & Marek Kucharzewski (2018). Honey as medicine: historical perspectives, Journal of Apicultural Research, 57: 1, 113.

Ashrafi S. Mastronikolas S. and Wu CD. Use of Honey in Treatment of Aphthous Ulcers IADR/AADR/CADR 83rd General Session. Baltimore, MD., USA: 2005. pp. 9–12.

Awasum, C. A., Fotzo, S. L. M., Ndukum, J. A., Genesis, C. D., & Zoli, A. (2015). Gas chromatography-mass spectroscopy analysis and chemical composition of Ngaoundere, Cameroon honey. American Journal of Bioscience and Bioengineering, 3(5), 33-36.

Bogdanov S. urendic T. Sieber R. and Gallmann P. Honey for nutrition and health: A review. J. Am. Coll. Nutr., 2008; 27: 677–689.

Bueno-Costa, FM. Zambiazi RC. Bohmer BW. Chaves FC. da Silva W. Zanusso, JT. and Dutra I. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil LWT - Food Sci Technol (Lebensmittel Wissenschaft -Technol) 2016; 65: 333-340. doi: 10.1016/j.lwt.2015.08.018.

Campeau, M. E., & Patel, R. (2014). Antibiofilm Activity of Manuka Honey in Combination with Antibiotics. International journal of bacteriology, 2014, 795281. https://doi.org/10.1155/2014/795281

Cantarelli, M.A., Pellerano, R. G., Marchevsky, E. J., & Camiña, J. M. (2008). Quality of honey from Argentina: Study of chemical composittion and trace elements. The Journal of Argentine Chemical Society, 96(1-2), 33-41.

DeMera, J. H., & Angert, E. R. (2004). Comparison of the antimicrobial activity of honey produced by Tetragonisca angustula (Meliponinae) and Apis mellifera from different phytogeographic regions of Costa Rica. Apidologie, 35(4), 411-417.

Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002; 8(9):881-890. doi:10.3201/eid0809.020063.

Ebenezer, I. O., & Olugbenga, M. T. (2010). Pollen characterisation of honey samples from North Central Nigeria. Journal of Biological Sciences, 10(1), 43-47.

Egan S. and Penesyan A. “Marine bacteria as a source of new antibiotics”. In: Bioactive Compounds: Types, Biological Activities and Health Effects; Nova Science Publishers, Hauppauge, New York, USA (2012): 381-412.

Hancock, REW. The end of an era?. Nature Reviews Drug Discovery 2007; 6: 28 https://doi.org/10.1038/nrd2223.

Haney, E. F., Mansour, S. C., Hilchie, A. L., de la Fuente-Núñez, C., & Hancock, R. E. (2015). High throughput screening methods for assessing antibiofilm and immunomodulatory activities of synthetic peptides. Peptides, 71, 276–285. https://doi.org/10.1016/j.peptides.2015.03.015

James, O. O., Mesubi, M. A., Usman, L. A., Yeye, S. O., Ajanaku, K. O., Ogunniran, K. O.,... & Siyanbola, T. O. (2009). Physical characterisation of some honey samples from North-Central Nigeria. International Journal of Physical Sciences, 4(9), 464-470.

Kasprzyk I. Depciuch J. Grabek-Lejko D. and Parlinska-Wojtan M. FTIR-ATR spectroscopy of pollen and honey as a tool for unifloral honey authentication. The case study of rape honey. Food Control. 2018; 84: 33–40.

Katz ML, Mueller LV, Polyakov M and Weinstock SF. Where have all the antibiotic patents gone? Nat Biotechnol. 2006 Dec; 24(12): 1529-31. doi: 10.1038/nbt1206-1529. PMID: 17160057.

Khan, F. (2014). Antimicrobial Properties of South African Honeys Against Pathogens Associated with Wounds (Doctoral dissertation, University of the Witwatersrand).

Khosravi, A. R., Shokri, H., Katiraee, F., Ziglari, T., & Forsi, M. (2008). Fungicidal potential of different Iranian honeys against some pathogenic Candida species. Journal of apicultural research, 47(4), 256-260.

Kostakioti M. Hadjifrangiskou M. and Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med. 2013; 3(4): a010306. doi: 10.1101/cshperspect.a010306.

Maddocks, S. E., Jenkins, R. E., Rowlands, R. S., Purdy, K. J., & Cooper, R. A. (2013). Manuka honey inhibits adhesion and invasion of medically important wound bacteria in vitro. Future Microbiology, 8(12), 1523-1536.

Mathur H. Field D. Rea MC. Cotter PD. Hill C. and Ross RP. Fighting biofilms with lantibiotics and other groups of bacteriocins. NPJ Biofilms Microbiomes. 2018; 4:9. doi: 10.1038/s41522-018-0053-6.

Mohanty SK. Mallappa KS and Godavarthi A. “Evaluation of antioxidant, in vitro cytotoxicity of micropropagated and naturally grown plants of Leptadenia reticulata (Retz.)Wight & Arn.-an endangered medicinal plant”. Asian Pacific Journal of Tropical Medicine. 2014; 7(1): S267–S271. DOI: 10.1016/S1995-7645(14)60244-3.

Perez, C. (1990). Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp, 15, 113-115.

Samarghandian S. Farkhondeh T. and Samini F. Honey and Health: A Review of Recent Clinical Research. Pharmacognosy Res., 2017; 9(2):121-127. doi:10.4103/0974-8490.204647.

Shirlaw, O., Billah, Z., Attar, B., Hughes, L., Qasaymeh, R. M., Seidel, V., & Efthimiou, G. (2020). Antibiofilm activity of heather and manuka honeys and antivirulence potential of some of their constituents on the DsbA1 enzyme of Pseudomonas aeruginosa. Antibiotics, 9(12), 911.

Stagos D, Soulitsiotis N, Tsadila C, Papaeconomou S, Arvanitis C, Ntontos A, Karkanta F, Adamou-Androulaki S, Petrotos K, Spandidos DA, Spandidos DA, et al: Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. International Journal of Molecular Medicine 2018; 42: 726-734. https://doi.org/10.3892/ijmm.2018.3656.

Velásquez, P., Montenegro, G., Leyton, F., Ascar, L., Ramirez, O., & Giordano, A. (2020). Bioactive compounds and antibacterial properties of monofloral Ulmo honey. CyTA-Journal of Food, 18(1), 11-19.