Riyadh Shamki Ali et al /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3111-3117

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Diagnosis of volatile components of local Iraqi honeys by GC- Mass spectrophotometry and study its effect against some pathogenic bacteria

[1]Riyadh Shamki Ali [2]Manal Abdalwahed Alssiraj [3]Laith Fareed Hasan Al-Obaidi
[1,3] College of Agriculture / Kufa University/Iraq, [2]College of Agriculture / Karbla University/Iraq

Abstract
Eight honey samples at four different concentrations (20%, 30%,40% and 50%) were selected to study its effect against five pathogenic bacteria (*Streptococcus pyogenes, E.coli, Proteus mirabilis, Staphylococcus aureus* and *Pseudomonas aerugenasa*).In addition,the volatile component in local honey samples of different vegetarian sources was investigated.The results indicated that all honey samples has an inhibition effect against all the selected pathogenic bacteria in this study.50% concentration for all honey samples has the highest inhibition against all selected pathogenic bacteria.Gas chromatography-mass spectrometry (GC/MS) revealed a total of 95 compounds for 8 types of honey. The chemical composition of the types of honey was very diverse, owing to the presence of compounds from different chemical types, for instance: alcohols, phenols, ketones, organic acids, esters and hydrocarbons (aliphatic, aromatic and cyclic). The performed data showed that the obtained volatile profiles of the studied types of honey differed and it was concluded that analysis of the volatiles could be effective for the characterization of the jars of honeys sources.

INTRODUCTION:

Honey is a natural product produced from nectar and perfusion of plants by honey bees, Natural honey has about 200 substances which have been mentioned, its Complicated not only of a high Concentrated solution of sugars, it also consists complex mixture of other saccharides, Amino acids, peptides, enzymes, proteins, organic acids, polyphenols, carotenoids, vitamins and minerals [1]. The Sugars are the main components of any kind of honey, Includes about 95% of its dry weight, the majority are glucose and fructose [2]. While proteins in honey are basically enzymes and it contains about 0.5% proteins and the protein contents in some honeys can be over 1 000 mg/g. The main enzymes include diastase, invertase, glucose oxidase and catalase, while the content of amino acids is small. It has been recorded that almost all of the essential amino acids present in honey [3].The mainly amino acid is proline, which represent about 50-85% of all the amino acids. The content of organic acids in honey is low and about 18 organic acids have been recorded [4]. A high percentage of acidity present in honey is added by honey bees Gluconic acid. Studies have recorded that a high range of trace elements is present in honey, consist of Al, Ba, Bi, Co, Cr, Mo, Ni, Pb, Sn, Ti. In addition to the minerals (Ca, Cu, Fe, K, Na, Mg, Mn, Zn), among its, main mineral is potassium while copper presents the lowest amount [5]. Vitamins like thiamin (B1), riboflavin (B2), pyridoxine (B6), and ascorbic acid (C) have been reported that their amount is very small in honey. When honey is treated with moderate heat or storage, a compositional change can appear due to crystallize of the carbohydrates, Millard reaction, and degradation of fructose in honey solutions [6]. Phenolic compounds are the highest types of phytochemicals, While plants containing phytochemicals can be used as a source nutrient of the bees. Bioactive compounds can be transferred to honey contains high different in contents of most phytochemicals according to floral. Honey produced by bees fed herbal extracts has recorded higher antioxidant activity than natural honey. A Honey has long researches of use as an active medicine for a high range of disease types. The biological ability of honey has been production of hydrogen peroxide formed by the enzyme glucose oxidase; antioxidant activity, low pH value; osmotic action, and a content of enzymes [7]. The antimicrobial mechanisms of honey are according to its high osmolality, acidity, content of hydrogen peroxide and non-peroxide, and antibacterial compounds such as flavonoids, lysozyme, and phenolic acids [8]. The level of hydrogen peroxide is appropriate relatively rang of glucose oxidase and the catalase source of pollen, at the same time not all types of honey have the same therapeutic active because large variation in its antibacterial effects. The variable antibacterial activity among honey depends on its floral [9]. Many studies have used GC mass spectrophotometer technique to diagnose a volatile and nonvolatile compounds of honey also the chemical components such as water, sugar, insoluble solids, acidity and enzyme activity such as diastasis was studied [10]. As for volatile compounds, many researchers found similar proportions in different types of honey with a slight variation in quantities. [11] estimated 110 volatile compounds in 43 samples of honey with the Trap-GC/MS method from various botanical and geographical origins. The authors found that the majority of study honey samples consist the following: aldehydes, ketones and short-chain alcohols. In addition, [12] used the SPME--GC/MS to study orange, eucalyptus and chestnut honeys, and confirmed the presence of 113 volatile compounds belonging to the following layers of compounds: acyclic and monocyclic monoterpenes and their oxygenated derivatives, furan, sulfuric derivatives, aliphatic and aromatic and nitrogenous compounds. The most popular of these components were present in almost studied samples and only some of them could be taken as potential markers of the botanical origin of a given type of honey. The aim of the current study was to identify the volatile honey components present in 8 types of honey (multiflora, heather, buckwheat, and lime-honeydew) using GC/MS techniques and study its effect against some pathogenic bacteria.

MATERIAL AND METHODS:

Honey samples collection

Eight types of honey produced from different areas of the middle Euphrates governorate regions were collected (Table 1). Honey samples were kept in glass containers at room temperature under sanitary conditions.

Table (1) Honey samples and source of bee feeding

Samples	Type of bee feeding
H1	*Zizpus spina* (Seder)
H2	*Medicago sativa* (Alfalfa)
H3	Eucalyptus
H4	Dubas bug
H5	Medicago sativa (Alfalfa)
H6	Zizpus spina (Seder)
H7	Eucalyptus
H8	Medicago sativa (Alfalfa)

Strains sources:

Five Bacterial isolates (*Pseudomonas aerugenasa, E.coli , Proteus mirabilis, Staphylococcus. Aureus* and *Streptococcus*

pyogenes) were obtained from hospitals in Najaf Governorate and it was diagnosed using VITIK2 systems.

Preparation of honey solutions:

Honey solutions were prepared immediately before using by diluting samples to the required concentrations (20, 30, 40, and 50) %.

Sensitivity test:

The isolates were cultivated into the center of the Marlinton agar by cotton swab using the streaking method by taking samples from the Nutrient broth and then examined the sensitivity by the Disk diffusion method to measure the resistance of pathogenic bacteria to 18 antibiotics against the positive and negative gram (Table 2). The samples were then incubated at 24 ° C for 24 h and antibiotic inactivation diameters were then recorded in special tables [13].

Table (2): Antibiotic symbols used and their concentrations

Number	Name of Antibiotic	Symbol	Conce
1	NORFLOXACIN	NOR	10mcg
2	Chloramphenicol	KF	30mcg
3	AMPICILLN	AM	10mcg
4	NEOMYCIN	N	30mcg
5	CEFTRIAXONE	CRO	30mcg
6	TETRACYCLINE	TE	30mcg
7	Levofloxacin	LEV	5mcg
8	CIPROFLOXAIN	CIP	5mcg
9	MASTDISCS	MEM	10mg
10	CLOXAOILLIN	CX	1mcg
11	RIFAMPAN	RA	5mcg
12	GENTAMICIN	CN	10mcg
13	AMIKACIN	AK	10mcg
14	CEFTRIAXONE	CI	30mcg/discs
15	TRIMETHOPRIM	TMP	5mcg
16	IMPENEM	IPM	10mcg
17	Vancomycin	VA	30c
18	CEFOXITIN	Fox	30mcg

Microbiological technique of honey against pathogenic bacteria:

The antibacterial studied were conducted according to the standard method [14]. Four wells of about 0.5 mm diameter were cut on agar-plate using a sterile cork borer, then honey was introduced into wells made in Mueller Hinton agar which previously inoculated with a single bacterium. Zones of inhibition were measured after 48h at the optimum temperature for each bacterium.

Limitation of bioactive compounds for honey samples by GC-MS spectrophotometer technique:

Bioactive compounds presented in the honey are diagnosed for each raw ethanol extract after it has been dissolved 0.1 g of each extract in 10 ml of 99% ethanol alcohol to get a solution that can be injected into a GC-MS device (QP2010, Shimadzu Company, Japan)[12] .The samples were examined in the economic laboratory, Ministry of Science and technology under the following circumstances: in auto-injector unit (ATUNE.U), Injection volume 5.00 uL, Viscosity Comp. Time: 0.1 sec, Pumping Times: five, Inj. Port Dwell Time: 0.2 sec, Washing Volume: 5uL, Solvent Selection: only C; In the GC-2010 unit, Column Oven Temp. : 40.0 °C, Injection Temp. 280.00 °C, Injection Mode :Split, Flow Control Mode : Pressure :7.037 psi , Total flow :34.0 mL/min, Column flow :1.00 mL/min, Linear Velocity :34.772 cm/sec, Purge flow :0.9 mL/min, Split Ratio :30.0,High Pressure Injection :OFF, Carrier Gas Saver :OFF, Splitter Hold :OFF, Oven Temp. Program Equilibrium Time: 1.0 min, [GC Program], [GCMS-QP2011 Ultra], Ion Source Temp: 250.00 °C, Interface Temp. :280.00 °C, Solvent Cut Time :3 min, Detector Gain Mode :Relative, Detector Gain :0.69 kV +0.10 kV, Threshold :0, If(--Group 1 - Event 1-Start Time :3.00min, End Time :15.00min, ACQ Mode :Scan, Event Time :0.30sec, Scan Speed :2877, Start m/z :20.00, End m/z :500.00. After it gets a mass spectrum of each compound, it is identified with curves separated by a NISRRTS7 database[15] .

Statistical Analysis

The results were expressed as mean values ± standard deviations. Significant differences between control and treatments were determined using Duncan's multiple range tests at a level of $P < 0.05$. Statistical analysis was performed using the statistical analysis system [16].

RESULTS AND DISCUSSIONS:

Effect of some antibiotics against some pathogenic bacterial

Eighteen antibiotics were selected to study the resistance of pathogenic bacteria against them. Table (3) showed the size of zone inhibition for some selected antibiotics against some pathogenic bacteria. The results indicated that there was a significant difference between antibiotics. CX, VA, RA, FOX, and TE did not show any inhibition zone against any of the pathogenic bacteria selected in this study. While other types of antibiotics showed an inhibition against some of the pathogenic bacteria. KF antibiotic showed an inhibition against *Proteus mirabils and E. coli* only (20 mm and 11 mm) respectively. However, another type of antibiotics showed an inhibition against all the pathogenic bacteria selected in this study such as AK, TMP, NOR, MEM and CN. The highest inhibition zone was 31 mm when TMP antibiotic used against *Staphylococcus aureus* while the lowest inhibition zone was 11 mm when KF antibiotic used against *E. coli* bacteria.

Effect of different concentrations of honey samples against pathogenic bacteria

Five strains of pathogenic bacteria were selected to evaluate the effect of honey on its growth. All honey varieties under study showed a varying inhibition effect on pathogenic bacteria. Table (4) showed the size of inhibition zone at different concentrations of honey samples against *Pseudomonas aerugenasa* bacteria .The result of the table indicated that there were no significant differences at concentrations of 20% and 30% for all honey samples except (H1) honey sample which showed a significant differences as the honey concentrations increased the size of inhibition zone increased. In addition, the same table showed that the highest inhibition zone was at (50%) honey concentration against *Pseudomonas aerugena*sa bacteria.

Table (5) showed the size of inhibition zone (mm) at different concentrations of honey samples against *Streptococcus pyogenes* bacteria. The results showed that there were a significant difference as the honey concentration increased the inhibition zone size increased for H1, H3, H5 and H6 honey sample. However, H2, H4 and H7 showed no significant differences in the concentrations of 20% and 30%. The highest inhibition zone against *Streptococcus pyogenes* was at a concentration of 50% for all honey samples except H2 samples.

Table (6) showed the size of inhibition zone (mm) at different concentrations of honey samples against *Staphylococcus aureus* bacteria. The results of the table indicated that there were no significant differences between a concentration of 20% and 30% of all honey samples against *Staphylococcus aureus* bacteria. In addition, the result showed that as the concentration of honey samples increased the inhibition zone increased. The highest inhibition zone was at a concentration of 50% of H2 and H5 samples (25mm and 25mm) respectively, while the lowest

inhibition zone was at a concentration of 20% and 30% for both H1 andH6 honey samples.

Table (7) mentioned the size of inhibition zone (mm) at different concentrations of honey samples against *E.coli* bacteria. The results of the table indicated that there were no significant differences between honey concentrations of 20% and 30% for the honey sample H1, H2, H4, H5 and H6. However, there was a significant difference between concentrations 20% and 30% of honey samples H3, H7 and H8. As the concentration of honey samples increased the inhibition zone increased. The highest inhibition zone was at a honey concentration of 50% for all honey samples which ranged between 16- 17 mm, while the lowest inhibition zone was at a honey concentration of 20% for both H1 and H6 sample.

Table (8) showed the size of inhibition zone (mm) at different concentrations of honey samples against *Proteus mirabilis* bacteria. The result indicated that there were no significant differences between concentration of 20% and 30% in H1, H3, H5, H6 and H8 honey samples. However, there was a significant difference between a concentration of 20% and 30% for H2, H4 and H7.The highest inhibition zone was at a concentration of 50% for both honey samples H2 and H4 (25mm and 25 mm) respectively, while the lowest inhibition zone was at a concentration of 20% for H5 sample (11mm).

Table 3: Size of inhibition zones (mm) for antibiotics used for bacteria isolates under study

Traditional Antibiotic	*Streptococcus pyogenes*	*E.coli*	*Proteus mirabilis*	*Staphylococcus aureus*	*Pseudomonas aerugenasa*
CX	Zero	Zero	Zero	Zero	Zero
AK	15 ± 1.7	15 ± 1.7	18 ±1.1	13 ± 1.5	19 ± 0.5
VA	Zero	Zero	Zero	Zero	Zero
RA	Zero	Zero	Zero	Zero	Zero
KF	Zero	11± 1.1	20± 1.7	Zero	Zero
Cl	Zero	Zero	Zero	Zero	Zero
FOX	Zero	Zero	Zero	Zero	Zero
TMP	29 ± 1.7	32± 1.1	27± 1.1	31 ± 0.5	28 ± 1.1
CIP	24 ± 1.1	15± 0.5	Zero	21 ± 0.5	13 ± 0.5
TE	Zero	Zero	Zero	Zero	Zero
LEV	17 ± 0.5	25± 1.1	Zero	19 ± 0.5	21 ± 1.1
NOR	24 ± 1.1	15± 0.5	20± 1.5	22 ± 1.7	17 ± 0.5
CRO	Zero	15 ± 0.5	Zero	Zero	Zero
MEM	22 ± 1.7	21 ± 1.1	21± 1.1	22 ± 1.7	22 ± 1.7
N	14 ± 1.1	15 ± 0.5	Zero	12 ± 0.5	11 ± 1.1
AM	13 ± 1.5	Zero	Zero	11 ± 1.1	Zero
CN	17 ± 0.5	12 ± 0.5	15± 0.5	15 ± 0.5	18 ± 2.3
LSD	2.7979	2.2043	2.1672	2.3466	2.5453
Significant level	0.05	0.05	0.05	0.05	0.05

Table (4) Size of inhibition zone in (mm) at different concentrations of honey samples against *Pseudomonas aerugenasa* bacteria

concentration / samples	20%	30%	40%	50%	Samples means
H1	9 ± 1.7	11 ± 1.5	13 ± 1.7	21 ± 2.6	13.5 ± 1.6
H2	11 ± 1.5	11 ± 1.5	15 ± 1.7	23 ± 2.3	15 ± 1.6
H3	11 ± 1.5	11 ± 1.5	13 ± 1.7	19 ± 1.7	13.5 ± 1.2
H4	11 ± 1.5	11 ± 1.5	15 ± 1.7	23 ± 2.3	15 ± 1.6
H5	11 ± 1.5	12 ± 1.1	13 ± 1.7	17 ± 1.1	13.5 ± 0.9
H6	9 ± 1.5	9 ± 1.5	14 ± 1.1	21 ± 0.5	13.5 ± 1.5
H7	9 ± 1.5	9 ± 1.5	14 ± 1.1	21 ± 0.5	13.5 ± 1.5
H8	14 ± 1.1	14 ± 1.1	17 ± 1.1	21 ± 0.5	16.5 ± 0.9
Mean of cons	10.52 ± 0.5	11.52 ± 0.5	14.12 ± 0.5	20.75 ± 0.6	20.52 ± 0.5

· LSD ≥ 0.03 for samples 2.2195 **· LSD ≥ 0.001 for conce 1.5964**

Table (5) Zone of inhibition in (mm) for the different honey concentration against *Streptococcus pyogenes* bacteria

concentration / samples	20%	30%	40%	50%	Samples means
H1	13 ± 1.7	15 ± 1.7	17 ± 1.7	22 ± 0.5	16.75 ± 1.2
H2	14 ± 1.1	15 ± 0.5	16 ± 1.1	11 ± 0.5	11.5 ± 1.8
H3	9 ± 1.1	12 ± 1.7	16 ± 1.1	19 ± 1.7	14 ± 1.3
H4	16 ± 1.1	16 ± 1.1	19 ± 1.7	21 ± 2.8	18 ± 0.9
H5	14 ± 1.1	18 ± 1.1	22 ± 1.7	27 ± 1.1	20.25 ± 1.5
H6	13 ± 0.5	15 ± 1.7	17 ± 1.1	22 ± 1.7	16.75 ± 1.1
H7	18 ± 1.1	18 ± 1.1	19 ± 1.7	23 ± 1.7	19.5 ± 0.8
H8	14 ± 1.1	17 ± 1.1	18 ± 1.1	24 ± 2.3	18.75 ± 1.2
Mean of cons	13.87 ± 0.6	15.75 ± 0.5	18 ± 0.5	19.87 ± 1.6	20.65 ± 0.5

· LSD ≥ 0.001 for samples 2.0236 **· LSD ≥ 0.001 for conce 1.4309**

Table (6) Zone of inhibition in (mm) for the different honey concentrations against *Streptococcus aureus* bacteria

concentration / samples	20%	30%	40%	50%	Samples means
H1	12 ± 1.7	12 ± 1.7	14 ± 2.3	23 ± 2.3	15.25 ± 1.6
H2	17 ± 1.7	18 ± 1.1	19 ± 1.7	25 ± 2.3	19.75 ± 1.2
H3	16 ± 1.7	16 ± 1.7	19 ± 1.7	21 ± 2.08	18 ± 1.007
H4	17 ± 1.7	18 ± 1.1	19 ± 1.7	25 ± 2.3	19.75 ± 1.2
H5	15 ± 1.7	15 ± 1.7	15 ± 1.7	22 ± 1.7	16.75 ± 1.1
H6	12 ± 1.7	12 ± 1.7	14 ± 1.1	23 ± 1.7	15.25 ± 1.5
H7	13 ± 0.5	14 ± 1.1	16 ± 0.5	19 ± 0.5	15.5 ± 0.7
H8	17± 1.1	17 ± 1.1	21 ± 0.5	24 ± 2.3	19.75 ± 0.7
Mean of cons	14.87 ± 0.6	15.25 ± 0.6	17.12 ± 0.6	22.75 ± 0.7	20.55 ± 0.5

· LSD ≥ 0.001 for samples 2.347 **· LSD ≥ 0.001 for conce 1.6596**

Table (7) Zone of inhibition in (mm) for the different honey concentration against *E. coli* bacteria

concentration / samples	20%	30%	40%	50%	Samples means
H1	8 ± 0.5	9 ± 1.7	11 ± 1.5	17 ± 1.7	11.25 ± 1.2
H2	11 ± 1.5	11 ± 1.5	14 ± 1.1	17 ± 1.7	13. 25 ± 0.9
H3	9 ± 1.7	12 ± 1.7	12 ± 1.7	17 ± 1.7	12.5 ± 1.1
H4	11 ± 1.5	11 ± 1.5	14 ± 1.1	17 ± 1.7	13. 25 ± 0.9
H5	11 ± 1.5	11± 1.5	11 ± 1.5	17 ± 1.7	12. 5 ± 1.03
H6	8 ± 1.1	9 ± 1.5	11 ± 2.3	17 ± 1.1	11.25 ± 1.2
H7	11 ± 2.3	13 ± 0.5	14 ± 1.1	17 ± 1.1	13.75 ± 0.8
H8	9± 0.5	11± 2.3	12 ± 1.7	16 ± 0.5	12 ± 1.0
Mean of cons	9.75 ± 0.5	10.87 ± 0.5	12.37 ± 0.5	16.87 ± 1.6	22.57 ± 0.5

· LSD ≥ 0.02 for samples 2. 1817 **· LSD ≥ 0.001 for conce 1.5427**

Table (8) Zone of inhibition in (mm) for the different honey concentrations against *Proteus mirabilis* bacteria

concentration / samples	20%	30%	40%	50%	Samples means
H1	12 ± 1.7	12 ± 1.7	15 ± 1.7	21 ± 2.08	15± 1.3
H2	14± 1.1	16 ± 1.1	16 ± 1.1	25 ± 2.3	17.75 ± 1.4
H3	14 ± 1.1	15 ± 1.7	16 ± 1.1	21 ± 2.08	16.5 ± 1.05
H4	14 ± 1.1	16 ± 1.1	16 ± 1.1	25 ± 2.3	17.75 ± 1.4
H5	11 ± 1.5	11 ± 1.5	15 ± 1.7	22 ± 1.7	14.75 ± 1.5
H6	12 ± 1.7	12 ± 1.7	15 ± 1.7	21 ± 0.5	15± 1.2
H7	12 ± 1.7	16 ± 0.5	17 ± 0.5	22 ± 1.7	16.75 ± 1.2
H8	13± 0.5	14 ± 1.1	15 ± 1.7	22 ± 1.7	16 ± 1.2
Mean of cons	12.75 ± 0.4	14 ± 0.5	15.62 ± 0.4	22.37 ± 0.6	21.65 ± 0.4

· LSD ≥ 0.02 for samples 2.1817 **· LSD ≥ 0.001 for conce 1.5427**

Table 9: Minimum Inhibitory Concentration (MIC) (mg/ml) for Iraqi honey samples

Honey sample	*Streptococcus pyogenes*	*E.coli*	*Proteus mirabilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
H1	8.6 ± 0.3	6.8 ± 0.6	8.3 ± 0.6	7.6 ± 0.8	9.5 ±0.8
H2	7.8 ± 0.6	9.7 ± 1.1	8.5 ± 0.8	6.4 ± 0.8	8.9 ±0.5
H3	9.6 ± 0.8	6.5 ± 0.8	10 ± 0.5	7.1 ± 0.5	8.5 ± 0.8
H4	10 ± 0.5	5.8 ± 0.5	8.9 ± 0.5	9.8 ± 1.1	7.9 ± 0.6
H5	6.5 ± 0.8	5.5 ± 0.5	7.3 ± 0.6	5.4 ± 0.8	10 ± 0.5
H6	9.3 ± 0.6	7.6 ± 0.3	6.1 ± 0.5	8.6 ± 0.3	7.5 ± 0.8
H7	8.5 ± 0.8	6.4 ± 0.8	8.4 ±0.3	6.4 ± 0.8	9.3 ± 0.6
H8	7.6 ± 0.8	6.3 ± 0.6	7.5 ±0.8	6.9 ± 1.1	8.5 ± 0.8
LSD	2.1834	2.246	1.9716	2.6378	2.2593
Significant level	0.05	0.02	0.02	No	No

From all the discussed table, we can say that the highest inhibition zone was at a concentration of 50%for all honey samples. The average inhibition zone of all types of honey samples at a concentration of 50% between (11-25) mm, this results consistent with a study conducted from [17] who found that the area of inhibition of various honey samples ranged between (15-30) mm. Moreover, [18] recorded that the inhibition activity of Nigerian honey samples ranged from (8-10) mm at 50%

concentration. Another study mentioned that honey's inhibition ability was (15-30) mm at 50% concentration; this is due to the inhibitory effect of honey. The high sugar content in the honey leads to osmotic and does not have a wet environment to grow bacteria as well because of high acidity and the present of the enzyme glucose oxidase, which produces hydrogen peroxide that killed bacteria [19].

The minimum inhibitory concentration (MIC) of honey samples under study against pathogenic bacteria :

Five strains of pathogenic bacteria were selected to evaluate the effect of honey on its growth using the alkaline dilution method to find the lowest inhibitory concentration of growth and compare it with the control formed from the tubes of the container in the middle of the plant with the honey and the positive control at the center of the plant with the bacteria and at the same concentrations. The results as shown in Table (9), Honey samples showed a differentiated tolerance to pathogenic bacteria that appeared to be a 5.4mg / ml of H5 sample toward *Staphylococcus aureus* to 10 mg/ml toward *Pseudomonas aeruginosa.*

Analysis of Honey samples by GC-MS.

The identified volatile components of 8 honey samples are presented in Table(10) and Fig (1-10), 95 compounds were identified.The identified compounds in honey samples belonged to different chemical classes as follows: alcohols: e.g. ethanol, 1-propanol, 2-methyl-,2,3-butanediol; phenols: e.g. phenol, 3,4,5-trimethyl-; ketones: e.g. acetone, acetophenone, butyrolactone; organic acids: e.g., acetic acid, butanoic acid; esters: e.g. ethyl acetate, methyl3-methyl-, furfural,; aliphatic hydrocarbons, some of these compounds had biological activity such as antibacterial, antifungal and anticancer [20 and 21].

Table (10) Analysis of honey samples by GC-MS.

Sample no	Peak no	Compound	Retention time(R.T)	Area %	Similarity%	Effectiveness
H1	2	4H-Pyran-4-1,2,3 dihydro-3,5-dihydroxy-6-methyl	15.214	18.05	90	Antimicrobial, Anti inflammatory
H1	3	5-Hydroxy methyl furfural	18.743	49.48	86	an antioxidant an anti-allergen an anti-sickling agent
H2	5	5-Hydroxy methyl furfural	19.157	47.68	94	an antioxidant an anti-allergen an anti-sickling agent
H2	3	Dihydroxyacetone	12.911	10.75	74	
H3	6	4H-Pyran-4-1,2,3dihydro-3,5dihydroxy-6-methyl	15.754	14.52	83	Antimicrobial, Anti inflammatory
H3	8	5-Hydroxy methyl furfural	18.954	36.58	90	an antioxidant an anti-allergen an anti-sickling agent
H4	2	Acetic acid	4.427	33.46	90	antibacterial
H4	4	5-Hydroxy methyl furfural	18.301	38.91	76	an antioxidant an anti-allergen an anti-sickling agent
H5	4	5-Hydroxy methyl furfural	19.573	44.72	94	an antioxidant an anti-allergen an anti-sickling agent
H5	5	5-Hydroxy methyl furfural	24.523	0.19	89	an antioxidant an anti-allergen an anti-sickling agent
H6	4	4H-Pyran-4-1,2,3 dihydro-3,5-dihydroxy-6-methyl	14.972	16.97	90	
H6	5	5-Hydroxy methyl furfural	18.695	56.01	94	an antioxidant an anti-allergen an anti-sickling agent
H7	4	4H-Pyran-4-1,2,3 dihydro-3,5-dihydroxy-6-methyl	14.702	15.22	83	Antimicrobial, Anti inflammatory
H7	5	5-Hydroxy methyl furfural	18.298	58.51	90	an antioxidant an anti-allergen an anti-sickling agent
H7	6	5-Hydroxy methyl furfural	24.477	0.23	83	an antioxidant an anti-allergen an anti-sickling agent
H8	3	4H-Pyran-4-1,2,3 dihydro-3,5-dihydroxy-6-methyl	15.253	13.61	74	Antimicrobial, Anti inflammatory
H8	4	5-Hydroxy methyl furfural	18.704	40.07	90	an antioxidant an anti-allergen an anti-sickling agent

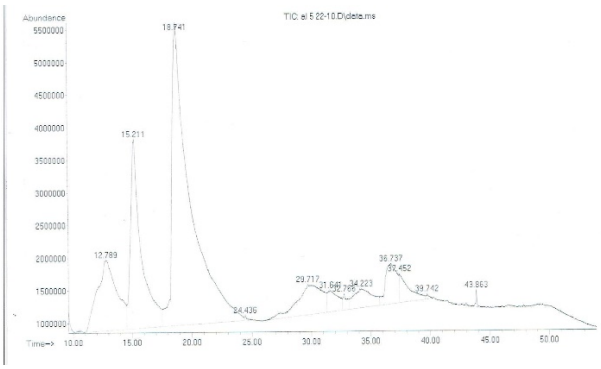

Fig 1Chromatogram of honey (1) by GC-MS.

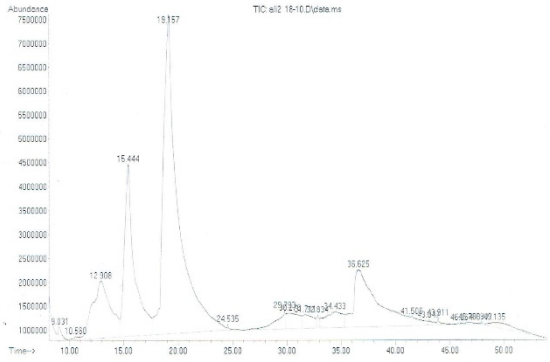

Fig2 Chromatogram of honey (2) by GC-MS.

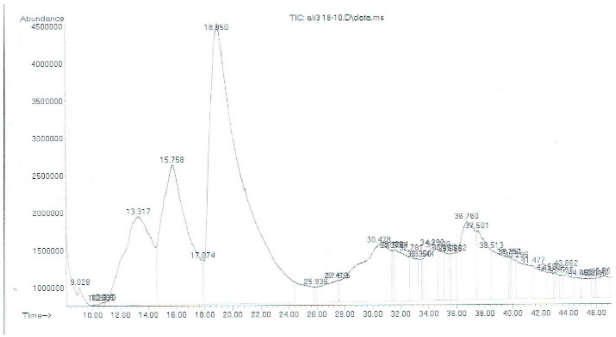

Fig3 Chromatogram of honey (3) by GC-MS.

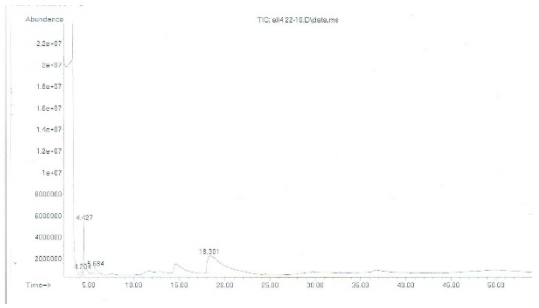

Fig4 Chromatogram of honey (4) by GC-MS.

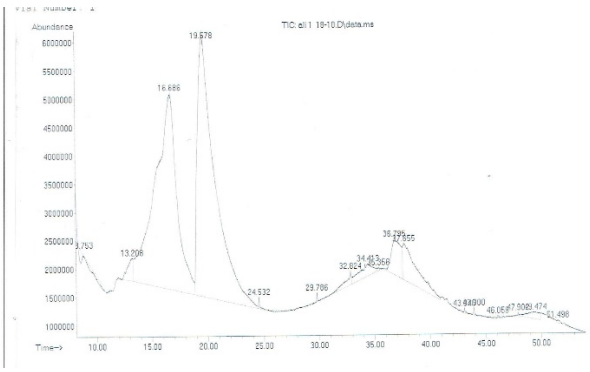

Fig5 Chromatogram of honey (5) by GC-MS.

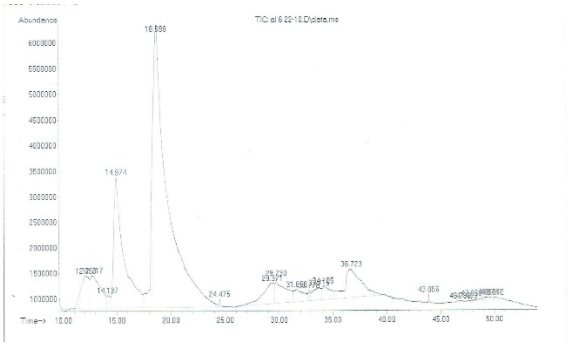

Fig 6 Chromatogram of honey (6) by GC-MS.

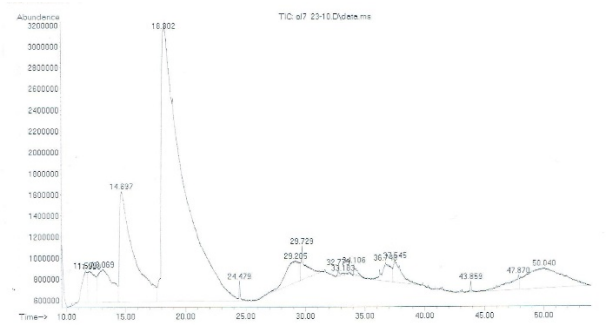

Fig 7 Chromatogram of honey (7) by GC-MS.

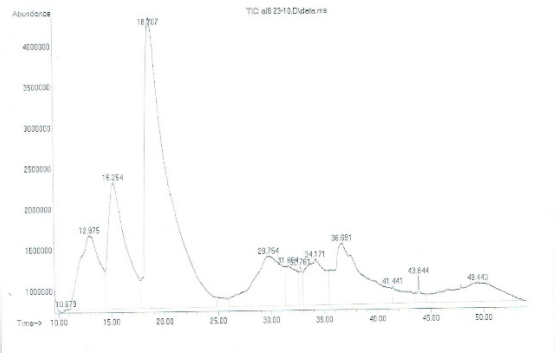

Fig 8 Chromatogram of honey (8) by GC-MS.

CONCLUSION:

In this study, the presence of all the above bioactive compounds in honey samples which is back to the inhibiting effect against some pathogenic bacteria. The variations in biochemical compounds content can be used to distinguish among honey sources. We should shed more light on some absent aspects of these chemical components as phenols, flavonoids, fatty acids… etc and its biological roles in human kind. One of the most important sides to be studied are the feeding types of bees and genetic analysis.

REFERENCES:

1- Alvarez-Suarez, J. M., Tulipani, S., Bertoli, S. R.-E., & Battino, M. (2010). Contribution of honey in nutrition and human health: a review. Mediterranean Journal of Nutrition and Metabolism, 3, 15-23.

2- Alissandrakis E., Dafera, D., Tarantilis P.A., Polissiou M., Harizanis P.C. (2003)– Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. Food Chemistry. 82, 575–582.

3- Azeredo, L., Azeredo, M., Souza, S., & Dutra, V. (2003). Protein contents and Physicochemical properties in honey samples of Apis mellifera of different Floral origins. Food Chemistry, 80, 249-254.

4- Nanda, V., Sarkar, B. C., Sharma, H. K., & Bawa, A. S. (2003). Physico-chemical properties and estimation of mineral content in honey produced from Different plants in Northern India. Journal of Food Composition and Analysis, 16, 613-619.

5- Stocker, A., Schramel, P., Kettrup, A., & Bengsch, E. (2005). Trace and mineral Elements in royal jelly and homeostatic effects. Journal of Trace Elements In Medicine and Biology, 19, 183-189.

6- Ball, D. W. (2007). The chemical composition of honey. Journal of Chemical Education, 84, 1643-1646.

7- Küçük, M., Kolaylı, S., Karaoğlu, Ş., Ulusoy, E., Baltacı, C., & Candan, F. (2007). Biological activities and chemical composition of three honeys of different Types from Anatolia. Food Chemistry, 100(2), 526-534.

8- Molan, P. C. (2009). Why honey works. In R. Cooper, P. Molan & R. White (Eds.), Honey in modern wound management. Aberdeen: Wounds UK Ltd.

9- Molan, P. C. (2001). Why honey is effective as a medicine. 2. The scientific Explanation of its effects. Bee World, 82(1), 22-40.

10- Bahaffi, S.O and AL-Lihabi, S.S. (2005) .Determination of volatile organic compounds in local honey by GAS chromatography –Mass spectrophotometer, Commun. Fac. Sci, Univ. Anik. vol(51) pp☹1-12).

11- Radovic, B. S., Careri, M., Mangia A., Musci , M., Gerboles, M., Anklam, E. (2001) . Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey. Food Chemistry. 72, 511 – 520.

12- Verzera, A., Campisi, S., Zappala M., Bonnaccorsi I. (2001). SPME-GC-MS Analysis of honey volatile components for the characterization of different floral origin. American Laboratory. 7, 18 – 21.

13- Alsirrag, M and Ali, R. (2018). Determination of antimicrobial and antioxidants activity of Salvia przewalskii seed oil against pathogenic bacteria and Fungi, The Sixth Scientific Conference "Renewable Energy and its Applications" IOP Publishing, Journal of Physics: Conf. Series 1032, pp: 1-8.

14- Lis-Balchin, M., S.G. Deans and Hart, S. (1996) .Bioactivity of commercial Geranium oil from different sources. J.Essent. Oil. Res. 8: 281-290.

15- Bentivenga G., D'Auria M., Fedeli P., Mauriello G., Racioppi R. (2004). SPME-GC-MS analysis of volatile organic compounds in honey from Basilicata. Evidence for the presence of pollutants from anthropogenic activities. International Journal of Food Science and Technology. 39, 1079 – 1086.

16- SAS, Statistical Analysis System. SAS User's. 2004, Statistics SAS Institute Inc. Editors, Cary. NC.

17- Iftikhar, F. (2010). Study Of Wound Healing and Antibacterial Activity of Bioactive Compounds Found in Honey from Different Flora of Pakistan. University Rawalpindi, Pakistan Registration No: 05-arid-31.

18- Osho, A. and Bello, O. O. (2010). Antimicrobial effect of honey produced by on some common human pathogens *Apis mellifera*.ASIAN J. EXP. BIOL. SCI. 1 (4) 2010:875-880.

19- Al-Khalissi, A.H.(2014). Study the inhibitory effect of the local honey against bacterial infections and its use in the treatment of burns and wounds and, MSC, AL-Mustansiriya University College of Basic Education.

20- Aljadi, A., & Kamaruddin, M. (2004). Evaluation of the phenolic contents and Antioxidant capacities of two Malaysian floral honeys. *Food Chemistry, 85(4),* 513-518.

21- Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G., & Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology, 46,* 3774-3779.