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Antibacterial Effect of Natural Honey of known active components by GC-MS technique against *Staphylococcus aureus*

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Abstract

Background: *Staphylococcus aureus* is a human pathogenic bacterium that colonizes the skin, oral cavity, upper respiratory tract, and gastrointestinal tract. It causes skin, soft tissue infections, and hospital-acquired infections. **Aim:** isolating *Staph. aureus* from skin infection and using honey as an alternative pathway to therapeutic therapeutic-resistant bacteria causing skin infection, as well as estimating the active compound by using the GC-MS Technique. **Methods:** (110) Clinical samples were collected from patients with skin infections through consultation clinics at Ibn Sina General Hospital of Mosul City/ Iraq, during the period from September 2024 until December 2024, by using conventional methods and the VITEK 2 system for identification. **Result:** The rate of isolation of *Staph. aureus* from skin infection was 76 (69.09%), but at the age group (11-30) years was 25% and (30-50) years was 40.7%, (51-70) years at 22.3% while at ages less than 10 years was 6.5% and ages more than 70 years was 5.2%. This bacterial species showed resistance against most of the 10 antibiotics under test using the disk diffusion method. Whole honey with its four dilutions (25,50,75, and 100%) gave an antibacterial effect against *Staph. aureus* growth. Gas Chromatography Mass Spectrometry (GC-MS) was used to investigate the biological effectiveness of honey and its (11) components, which have antibacterial effects.

Keywords: *Staphylococcus aureus*, skin infection, antimicrobial agent, compound of honey.

Introduction

The *Staphylococcus aureus* is a Gram-positive, spherical bacterium, it has grape–like clusters, it as golden yellow colonies on media, and it can produce hemolytic enzymes on blood agar media (1,2). It is typically found in the body as natural flora, causing nosocomial infection, and is considered a major cause of community–acquired infection. *It* colonizes mainly in skin, the oral cavity, the upper respiratory tract, and the gastrointestinal tract (3). It can cause several disorders, including infection of the skin and soft tissue infection, as well as bloodstream, bone, and endocarditis. It can create biofilm, which helps in adhering to different surfaces, especially on many medical devices in hospital settings (4,5). Independent of the patient's age, climate, or geographic location and some of the toxins are responsible for the primary clinical symptoms of skin disease by secretion of enterotoxins, exfoliations (ETs), Panton-Valentine Leukocidin, and Toxic Shock Syndrome Toxin1(TSST-1) (6). *Staphylococcus aureus* possesses several virulent factors like polysaccharides, capsules, toxins like leukocidins, Hemolysins, biofilm formation, adhesions, Peptidoglycan teichoic acid, enzymes,

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Protein A, and these contribute to increase of pathogenesis of *it* (7). The major problem of word wide it is control of antibiotic-resistant bacteria, and β –lactam antibiotics are important class of antibiotics employed for treatment mainly Gram-positive bacteria, yet to current development of broad – spectrum β –lactam antibiotics that ability to effect on various types of Gram-negative bacteria which lead to increase their benefit (8,9). *Staph.aureus* possess resistance to β –lactam antibiotics same as penicillin which have two resistance strategies against of β –lactam antibiotics, one of them it is the expression of β–lactam enzyme which it results of destroy of β –lactam antibiotics via hydrolysis it, and second mechanism it activation of the *blaZ* gene that encoded via the *blaZ* gene and position on transposable part of the large plasmid on the bacteria cell (10,11). Therefore, these genes are easily moved between bacterial cells by bacterial conjugation or horizontal gene transfer, like Methicillin resistance *S. aureus* (MRSA), which results from the gain of the *mecA* gene, which encodes penicillin–binding protein 2a (12,13). Because of the random use of antibiotics, which leads to the prevalence antibiotics antibiotic-resistant bacteria, and outcome increased rate of mordantly and morbidity among people and patients in hospitals, Therefor, must present an alternative solution for this problem by taking natural antimicrobial agents like plants or other compounds (14,15).

Honey is one of the most ancient substances that has been utilized as an antibacterial agent since ancient times. It is made by honeybees, which typically convert and process nectar collected from flowering plants. It is a sweet and delicious natural product that is consumed due to its high nutritional significance and impact on human health and medicine. Honey has antioxidants, antibacterial, anti-inflammatory, and antimicrobial properties in addition to its ability to heal wounds and burns (16,17). Many studies have displayed that honey can be used to treat several diseases like burns and wounds as well as ulcers, gastritis, stomach, and liver disease, Honey can also occasionally be used via combination with certain herbal products to treat cough, throat, and mouth problems, particularly with upper respiratory tract infection (18). The probable antimicrobial of honey is related to its physical and chemical properties, such as low pH, low protein content, high osmotic pressure, and high viscosity (19,20). Honey is composed of approximately 181 substances, the most of these substances is sugar it includes 38% fructose, 31% glucose, 7.3% maltose, 1.3% sucrose while the water contains present about 17%, So, because of its high sugar content, honey has a high osmotic pressure, which thought to be an antibacterial factor (21,22). The structure of honey varies greatly from one type to another and depends primarily on its plant source, season, and environmental factors. These factors can also support the biological effects. Much research has shown that the antioxidant capacity of honey is strongly related only to the concentration of the types of compounds, like the color, as dark honey is reported to contain a higher total phenolic content and therefore a higher antioxidant capacity (23,24). Therefore, the aim was to investigate the biological activity of the honey and use it as an alternative pathway to therapeutically resistant bacteria and estimate the active compound by using the GC-MS technique.

Material and method

Collection of samples:

Clinical Swabs (110) were collected from patients with skin infections through consultation clinics at Ibn Sina General Hospital of Mosul City/ Iraq, during the period from September to December 2024. The swabs were inoculated in a transport medium and cultured on blood agar and Mannitol Salt agar (Himedia – India) and incubated at 37°C for 24 hours. All isolated pure colonies were

diagnostic depending on Phenotypic (morphological, biochemical, and physiological characteristics) and confirmed by the VITEK 2 System.

Preparation for Honey:

The honey samples were taken from the market and stored in a sterilized, tightly screwed container in a dark, dry, and cool area. The sterile mesh was used to filter the honey to eliminate any refuse and keep it. The concentration of the honey solution at (25%, 50%, 75%, and 100%) was determined.

Antibiotic Sensitivity Test

The antibiotic susceptibility test was achieved depending on the Kirby–Bauer method (25), using Muller-Hinton agar medium, and ten antibiotic disks. The diameter of the inhibition zone around discs depended on the Clinical Laboratory Standards Institute (CLSI) guidelines.

Antibacterial Activity of the Honey

The potential activity of honey was determined according to (10,26). The surface of a Mueller-Hinton agar plate (Himedia-India) was inoculated with a suspension of bacterial growth compared with the standard McFarland tube, No.0.5 equals 1.0x108 CFU/ml. Lastly, a sterile corn borer with a size 6 mm diameter was used to cut five wells on the agar, Each plate's four wells were then filled with honey solutions at 200µl of dilution respectively and 200µl of distal water was placed in the central well as control and incubated plates at 37C for 24 hour.

Gas Chromatography–Mass Spectrometry (GC-MS) Technique:

The active compound of the honey was determined by using the GC–MS technique, which was applied for the identification of an Unknown compound in a honey solution that has antibacterial activity, and the result was compared with the standard compound in the library of the National Institute of Standards and Technology (NIST).

Result

Isolation and Identification

In the present study, the isolation rate was 76(69.09%) shown in Table 1. The bacteria isolates were identified based on phenotypic examination as Gram-positive cocci in a manner similar to grape clusters, as well as biochemical tests such as the oxidase test, catalase test, coagulase test, gelatinase, and motility. In addition to being able to grow on manntiol salt agar medium, they exhibit as large, round, golden, yellow-colored colonies and β-hemolytic on blood agar medium also tolerance the salt concentration of 10%, 15% NaCl, shown in Table 2 However, ten isolates that were confirmed based on VITEK 2 compact system.

The prevalence of *S. aureus* in clinical samples according to the age groups

Staphylococcus aureus was isolated in all age groups, the age 31-50 years showed the highest rate of isolation with 40.7% followed with 11-30 years at 25% while the age 51-70 years shown an isolation rate at 22.3%, and age less than 10 years and more than 70 years old showed low isolation rate at 6.5 % and 5.2% respectively shown on Table 3.

The Prevalence of *S.aureus* in clinical samples depending on the skin infection

On Table 4 shown the isolated bacteria from different skin infection, the result gave highest rate for isolation bacteria it was from the infection after surgical skin operation at 39(51.3%), followed by infection wound at 13(17.1%), Impetigo at 10(13.2.%), secondary infection burns at 6(7.9%), Folliculitis at 5(6.5%), and cellulitis at 3(4%).

Table1. The Percentage Isolation of *Staphylococcus aureus* bacteria in the current study

No. of sample	Positive isolation	Negative isolation
110	76 (69.09%)	34 (30.90%)

Table2. Identification of *Staphylococcus aureus* depends on Morphological, Biochemical tests, and growing on culture media.

	No. of *Staphylococcus aureus*									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Gram stain	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**	**Gram + purple cocci**
Oxidase test	-	-	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+
Coagulas-e test	-	+	-	+	+		+	+	+	+
Gelatinas-e test	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-
Growth on Mannitol salt agar	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**	**golden yellow colony**
Type of hemolysis on Blood agar	**α-hemol-ysis-**	**β-hemol-ysis**	**β-hemolys is**	**β-hemol-ysis**	**β-hemol-ysis**	**α-hemolys is**	**α-hemol-ysis**	**α-hemol-ysis**	**β-hemol-ysis**	**β-hemol-ysis**
tolerance NaCl 10%	+	+	+	+	+	+	+	+	+	+
tolerance NaCl 15%	**Weak**	+	+	+	+	**Weak**	+	**Weak**	+	+

+: Positive , - Negative

Table3. The Percentage Isolation of *Staphylococcus aureus* from Skin Infection according to Age

Age group	Number of Isolates	Percentage of isolates %
<10	5	6.5%
11- 30	19	25%
31- 50	31	40.7%
51- 70	17	22.3%
>70	4	5.2%
Total	76	100 %

Table4. Illustration of *Staphylococcus aureus* isolating from a skin infection

No	**Disease**	**Number of isolating *Staphylococcus aureus***	**Percentage**
1	**Infection wound**	13	17.1%
2	**Impetigo**	10	13.2%
3	**Secondary infection burns**	6	7.9%
4	**Folliculitis**	5	6.5%
5	**After surgical skin operations**	39	51.3 %
6	**Cellulitis**	3	4%
	Total	76	100%

Antibiotic sensitivity test

In this study, a sensitivity test was performed for 10 antibiotics. These antibiotics that are commonly used to treat *S. aureus* infections, the result showed that most bacteria isolates have a multidrug resistance pattern. All isolates appear 100% resistant to Ampicillin and resistant at 70%, 60% for Gentamicin and Tetracycline, respectively. The resistance rate of the isolates for Erythromycin and Cefixime showed the same ratio at 50%, and the resistance rate of Cefotaxime was 60%, while Clindamycin, Doxycycline, and Vancomycin antibiotics had the same percentage at 20%. On the other hand, Meropenem was the best antibiotic that affected on *S.aureus* at 100% also Clindamycin and Vancomycin antibiotics at the same result at 80 %. In addition, Erythromycin and Cefixime have the same result at 50 %, while Tetracycline and Cefotaxime have the same result at 40%. Consequently, the results showed that most *S. aureus* isolates under this study indicated high resistance to many types of antibiotics, as shown in **Figure 1**.

The Antibacterial Activity of the Honey

The biological effect of the honey was applied according to (19), the result showed that higher concentration of honey (100% and 75% were more effective than other concentration of honey, the isolates *S. aureus* 3 and *S. aureus* 10 were most isolates effected with honey concentration 100% and recorded same effected an diameters inhibition zone at 26mm, while isolates *S. aureus* 1, *S. aureus* 7 were recorded inhibition zone at 25mm for each them and isolates *S. aureus* 2, *S.aureus* 4, *S. aureus* 6 were recorded same inhibition zone diameter as 22mm, in addition, the isolates *S. aureus* 8, *S. aureus* 9, *S. aureus* 5 appeared inhibition zone at 21,20,19 mm respectively. While the diameters inhibition zone for concentration of honey of 75% for the isolates *S. aureus* 3,10,7 was recorded same effected at 22mm, *S. aureus* 1at 21mm, *S. aureus* 2, 8 with same effected at 17mm, *S. aureus* 4 at 16mm, *S. aureus* 6 at 15mm, *S. aureus* 9 at 14mm and *S. aureus* 5 at 12mm that. The middle concentration of honey 50% was given the inhibitory values for isolates *S. aureus* 3 at 19mm and less affected for other bacterial isolates. However, the inhibitory values for the low concentration of honey 25% were recorded as low diameter inhibition zones for all isolates, and all these results are shown in Table 5.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

On Table (6) appear the chemical compounds of honey with biological activity were identified using Gas Chromatography Mass Spectrometry(GC-MS), on our study (11) compound were identified and highest peak area of the honey was (22.376) for compounded β-Sitostero, 3-hydroxysteroids, i-Propyl 9octadecenoate(Z1-methylethyl ester, n-Propyl 9-octadecenoate.All compounds and formulas are shown in **Figures 2 and 3.**

Figure1. The result of the antibiotic susceptibility test for *Staphylococcus aureus*

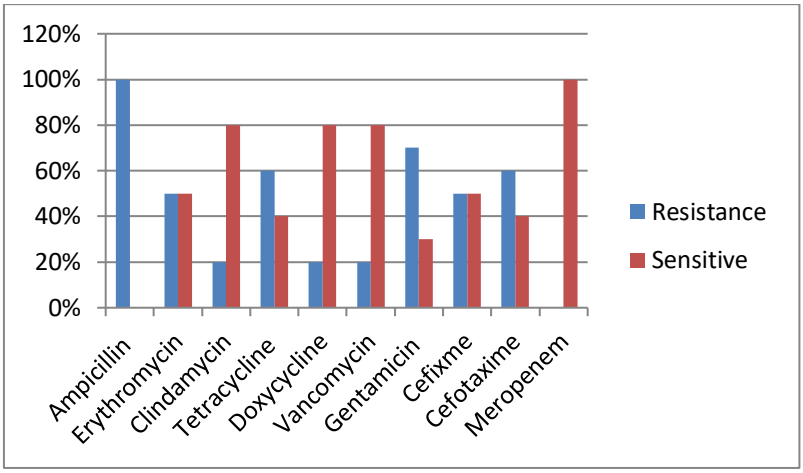


Table 5. The antibacterial activity of the honey against *Staphylococcus aureus*

NO. Isola,tes	The average of the inhibition zone diameter (mm) of the honey effect on *Staphylococcus aureus*			
	100%(v/v)	75% (v/v)	50%(v/v)	25% (v/v)
Staphylococcus aureus 1	25	21	16	11
Staphylococcus aureus 2	22	17	12	9
Staphylococcus aureus 3	26	22	19	15
Staphylococcus aureus 4	22	16	11	7
Staphylococcus aureus 5	19	12	7	---
Staphylococcus aureus 6	22	15	8	---
Staphylococcus aureus 7	25	22	17	10
Staphylococcus aureus 8	21	17	8	----
Staphylococcus aureus 9	20	14	8	7
Staphylococcus aureus 10	26	22	17	12

----, no effect

Table 6. The chemical composition of the Honey analysis by GC – mass

No	Retention time	Area %	Molecular Wight	Formula	Identified compound
1	2.102	0.22	84	C4H4O2 C5H8O	2(5H)-Furanone , .gamma.-Crotonolactone , .gamma.-Hydroxycrotonic acid lactone , .delta.,.alpha.,.beta.-Butenolide , Butenolide :2-Butenal, 2-methyl- , Crotonaldehyde, 2-methyl- , 2-Methyl-2-butenal , 2-Methylcrotonaldehyde , 2,3-Dimethylacrolein , 2-Me
2	20.503	14.27	256	C16H32O2	Hexadecanoic acid , Hexadecanoic acid , n-Hexadecoic acid , Palmitic acid, Pentadecanecarboxylic acid , 1-Pentadecanecarb
3	22.376	81.01	414.7	C29H50O	β-Sitosterol
4	22.498	3.66	324	C21H40O2	-Propyl 11-octadecenoate**,** Octadecenoic acid
5	24.740	0.02	169	C10H19NO	Propanamide, 3-cyclopentyl-N-ethyl-
6	25.043	0.07	129	C3H3N3O3	Furazan, methylnitro-
7	25.660	0.12	180	C13H24	1-Tridecyne , 1-C13H24
8	26.085	0.08	259	C8H14BrN5	Piperazine, 1-(3-bromo-1-methyl-1H-1,2,4-triazol-5-yl)-4-methyl
9	26.523	0.14	1141	C13H28OSi	Dihydromyrcenol, trimethylsilyl ether
10	27.631	0.16	300	C15H29N2O2P	Cyclooctyl N,N-diisopropylphosphoramidocyanidate Cyclooctyl diisopropylamidocyanidophosphate
11	29.575	0.20	456	C31H52O2	Cholest-5-ene, 3-(1-oxobuthoxy)- , Cholest-5-en-3-yl butyrate, Cholest-5-ene, 3-butanoyloxy- ,3-(Butyryloxy)cholest-5-ene , 100

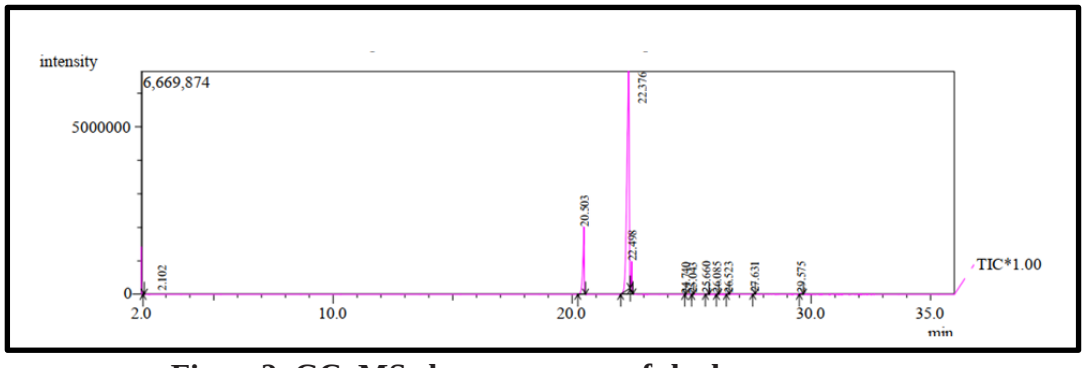
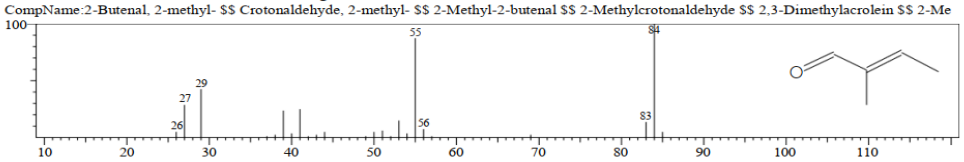
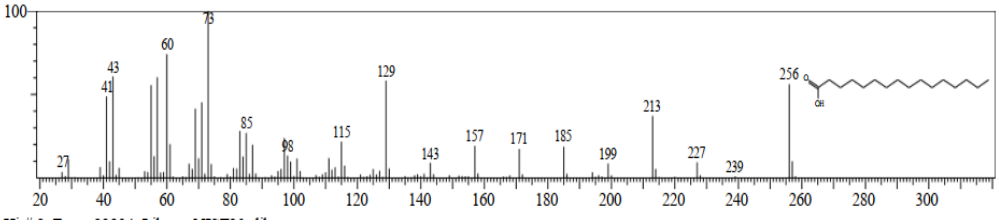


Figure2. GC–MS chromatogram of the honey

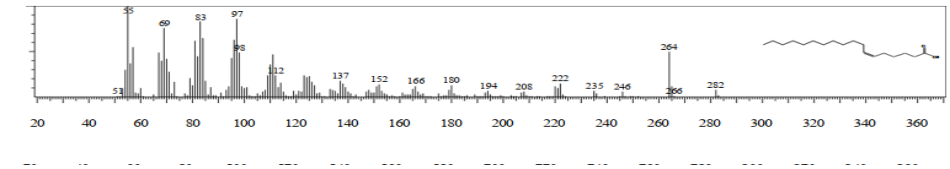
Hit#:2 Entry:906 Library:NIST20s.lib
SI:86 Formula:C5H8O CAS:1115-11-3 MolWeight:84
CompName:2-Butenal, 2-methyl- $$ Crotonaldehyde, 2-methyl- $$ 2-Methyl-2-butenal $$ 2-Methylcrotonaldehyde $$ 2,3-Dimethylacrolein $$ 2-Me



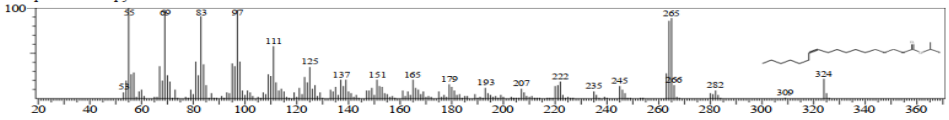
Hit#:1 Entry:31607 Library:NIST20s.lib
SI:71 Formula:C16H32O2 CAS:57-10-3 MolWeight:256
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecanecarb



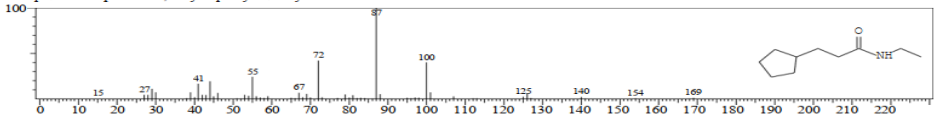
Hit#:1 Entry:142131 Library:NIST20-1.lib
SI:87 Formula:C29H50O CAS:38-46-5 MolWeight:414.7
Comp Name: β-Sitosterol



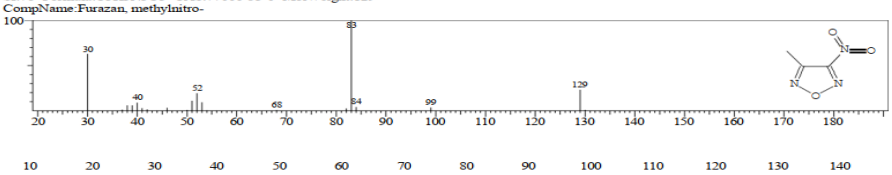
Hit#:1 Entry:191774 Library:NIST20-1.lib
SI:81 Formula:C21H40O2 CAS:0-00-0 MolWeight:324
CompName:i-Propyl 11-octadecenoate



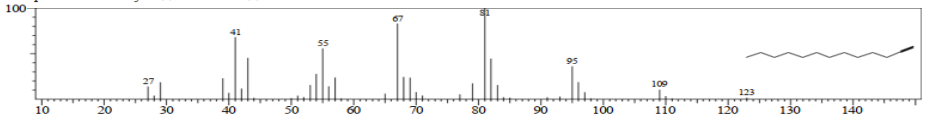
Hit#:1 Entry:28980 Library:NIST20-1.lib
SI:76 Formula:C10H19NO CAS:0-00-0 MolWeight:169
CompName:Propanamide, 3-cyclopentyl-N-ethyl-



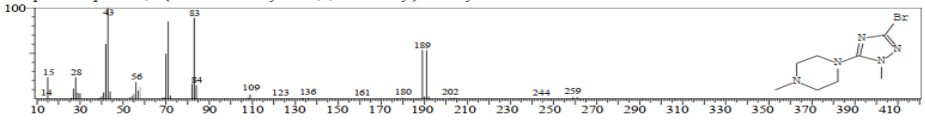
Hit#:1 Entry:8133 Library:NIST20-1.lib
SI:78 Formula:C3H3N3O3 CAS:77666-53-6 MolWeight:129
CompName:Furazan, methylnitro-



Hit#:1 Entry:18455 Library:NIST20s.lib
SI:73 Formula:C13H24 CAS:26186-02-7 MolWeight:180
CompName:1-Tridecyne $$ 1-C13H24 $$



Hit#:1 Entry:114580 Library:NIST20-1.lib
SI:52 Formula:C8H14BrN5 CAS:0-00-0 MolWeight:259 RetIndex:1854
CompName:Piperazine, 1-(3-bromo-1-methyl-1H-1,2,4-triazol-5-yl)-4-methyl-



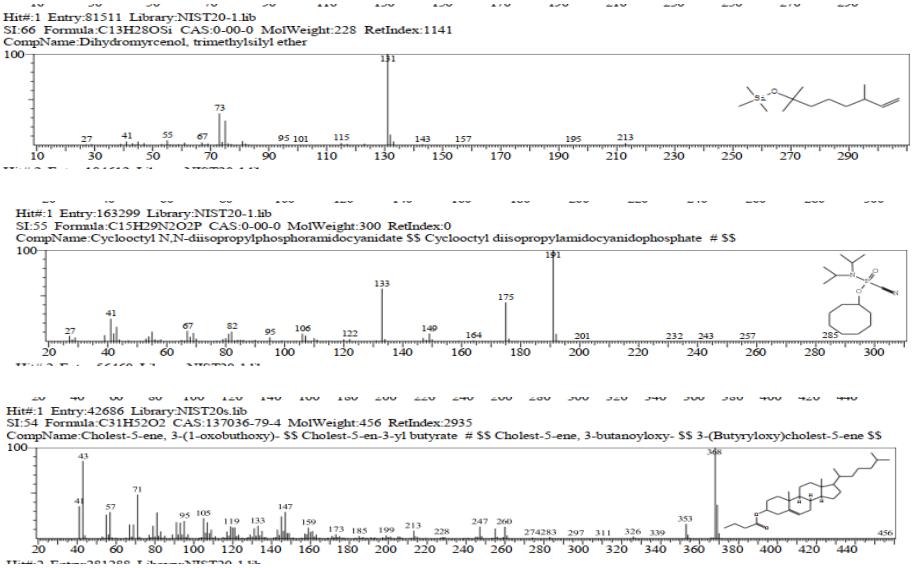


Figure 3. Chemical structure of the major identified compound of the Honey by the GC-MS Technique

Discussion

S. aureus is recognized as part of the normal flora colonies of the human skin, but some strains of these bacteria are considered a major reason for human infection, like skin lesions, soft tissue infections, foodborne illness, and gastroenteritis (27). In this study, about 110 samples were collected from skin infections, and only 76 (69.09%) isolates were diagnosed as *S. aureus*, and this result agrees with (28). All isolates of *S. aureus* were identified via phenotypic and physiological characteristics, appeared as cocci with diploids or tetrads, and accumulated in grape-like clusters with a purple color. *S. aureus* capability to ferment the mannitol sugar. So, when growing on Mannitol Salt Agar, the colonies appeared as golden yellow after incubation at 37 °C for 24 hours, which these due to the fermentation of mannitol sugar (29). *S.aureas* can ability to produce catalase enzyme and used to different between pathogen bacteria types, these enzyme extracellular generate from aerobic bacteria, *S. aureus* created hemolysin enzyme and produce β- hemolysis on blood agar plate with smooth yellow or white colonies, *S. aureus* was tolerant growth with 10 % NaCl and gave large, dark, convex, slightly colony with size (2-3mm) but showed weak growth on 15% NaCl and this result demonstrated by (30). Ten *S. aureus* isolates were confirmed using VITEK 2 compact system. This technique was characterized as a rapid and accurate test exploited for the detection of bacteria, the result was identical with the previous test and confirmed with ID message assurance level ranging from acceptable to excellent probability percentage from 94% - 99% (31).

The prevalence of *S. aureus* in clinical samples according to the age groups

S. aureus was isolated at the highest percentage at the age range 31-50, at 23% and these results agree with the current study, which demonstrated the isolation *of S. aureus* in different age groups, and the highest rate of isolation was recorded at ages 31-50 in 40.7%. *S. aureus* can colonies human and animal bodies, so they can cause various infections in different ages, and it is considered a zoonotic pathogen (32). Additionally, it was widespread

among the population**,** and thought it be one of the most common types of bacteria for patient–to–patient transmission, due to loose general rules of hygiene, which are important factors for transport and contamination with *S. aureus*. Therefore, it was a primary pathogen of global public health concern, and it ranked third in the world among reported foodborne pathogens (33-36).

The prevalence of *S. aureus* depends on the skin infection

S aureus is an infectious pathogen that causes various diseases, spectrum from skin infections to chronic pneumonia and bacteremia (37)**.** *S. aureus* was an important reason for skin infection, causing boils, abscesses, and infection on soft tissue (38), as well as deep infection like osteomyelitis, endocarditis (27). In addition, it was an essential pathogen in surgical wounds and a cause of nosocomial hospital and community–acquired infection (26). Was proved isolation *S. aureus* in high percentage from surgical skin and these result is agree with present study for found *S. aureus* at 39(51.3%) after surgical skin operations (30), also similar approximately result for that isolation *S.aureus* from skin infection report (30), and with (32) that showed *S.aureus* predominant pathogen that found after on surgical site infections (26). However, another study that refers to *S. aureus* infection has been documented as the most common pathogen causing hospital-acquired infection in Karama and Medical City of Baghdad (33).

Antibiotic sensitivity test

Based on the antibiotic sensitivity test, *S. aereus* isolates appeared multidrug resistant in our study, it resistant to Ampicillin at 100% and similar to the study of (38). A study showed *S. aereus* appeared resistant to Erythromycin, and it is an approach to our study that *S. aureus* has resistance to Erythromycin in 80% (39). Furthermore, some of *S.aureus* isolates showed a low rate of resistance to Vancomycin at 20% compared with the study of (40), which demonstrates none of the *S.aureus* isolates have any resistance to Vancomycin antibiotics agent. *S. aureus* in the current study showed high sensitivity for clindamycin, and this result agreed with (30)**.** Our results were compared and agree with (41), who isolated *S. aureus* from skin infections of patients attending General Samara Hospital in Salah El-Din and recorded a high sensitivity percentage for Imipenem and Vancomycin. Another study that also supports our results was recorded by (26) that isolated S. *aureus* from skin and soft infections, showing a high rate of sensitivity to Vancomycin, Erythromycin, Tetracycline, and Gentamycin. *S. aureus* in current study have high susceptibility to Doxycyclin and it compared with study of (42), interesting *S. aureus* appear a dynamic of horizontal transfer of antibiotics resistance gene from multi – drag resistance to other Staphylococcus species which leading to gave bacteria properties the resistance to antibiotics and most strain of *S. aureus* carrying genes that encode rang of virulent factor such as enterotoxins and antibiotics resistance (43-45) *S. aureus* have many of virulent gene and it integration with mobile genetic element like plasmid and transposon that result the rapid transfer of genetic materials between bacteria (46-48).

Antibacterial Activity of Honey

Many studies around the world refer to honey as having antimicrobial activity, especially against pathogenic bacteria. Therefore, the current study showed that the well diffusion method proved that honey possesses the highest significant effectiveness against *S. aureus*, and these results were in accordance with (14,49). These antibacterial activity of honey due to many chemical properties like as rising osmotic pressure, low pH, decrease protein contains, present tetracycline derivatives, vitamins and sugar as amylase, glucose, organic acid, ascorbic acid, benzoic acid, flavonoids, carotenoid, terpenes, lysozyme, H_2O_2 and other antioxidant factors

(11,12,16,48), the high concentration of honey (100%) was gave the beast result for inhibition *S. aureus* due to the honey is a saturated solution of sugar and low percentage of free water in honey makes it an insufficient and unfavorable environment for support growth bacteria, this free water is know the water activity (a_w) therefore, the honey has low water activity (a_w) that help for inhibition of growth bacteria, as well as the honey is a saturated solution of sugar, all these factors assisted to prevent bacteria growth (50-52). Many factors affect honey activity, like environmental conditions and the geographic location of the floral source. In addition, the years and time when honey was collected as well as the kind of bee that produces the honey, So, all elements were influential on the potential activity of the honey for inhibition of bacteria (53).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

In our study, the modern analysis technique was applied to the investigation of compounds of honey by GC–MS in Figure 3. Concomitantly, (54) stated that GC–MS analysis of the honey found many bioactive compounds, and these agree with the current study, which identified 11 chemical compounds. These compounds are related to fatty acids as well as volatile compounds and are Carbonyls, Aromatics, Alcohols, and esters' derivatives; however, most of these compounds act as antibacterial and antifungal agents. One of the compounds identified from honey was Furanon, it was polymeric biomaterial, and it has antimicrobial activity by inhibiting bacteria adhesion and slime layer production, which assists in limiting infection and controlling disease [55]. In addition, we detected Butenal, which has antibacterial effects. It binds with the cell wall bacteria and forms a complex with extracellular protein, leading to the destruction of the cell wall bacteria. Our result agrees with (56), which proved the antibacterial effects of Butenal on *E. coli, Klebsiella, and Staph.aureus, Pseudomonas aeuroginosa*. Hexadecanoic acid is another compound found in the honey also have potential antimicrobials effective (57), it occur by inhibition of enzyme and nucleic acid, protein synthesis as well as interference with cell membrane of bacteria, In addition to the reaction between hexadecenoic acid with hydroxyl group in lipopolysaccharide that causing of the change of structure in lipopolysaccharide which leading to cell wall become asymmetrical and lysed due to unbalance disruption of lipid on cell membrane (58). Sitosterol is a bioactive substance that is usually derived from plant cell membranes. It has antibacterial activity against *S. aureus* and *E. coli* (59). Sitosterols have anti-inflammatory effects and assist in wound healing and the treatment of skin infections (60). A study by (61) mentions the effect of Sitrosterol on Gram-positive bacteria, and it was more susceptible to antibacterial agents due to the composition of the cells, which are more polar, having a single–layer peptidoglycan and low lipid in structure. Sitrosterol also has antioxidant effectiveness. In the current study, Octadecenoic acid was detected it is derived from unsaturated fatty acid and has effectiveness as an antibacterial and antifungal agent, and it has antibiofilm and antioxidant properties against *S. aureus* (62,63). The other compound is Propanamide, which has bioactivity against *S. aureus* (64). Propanamide derivatives also have a good potential effect as an anticancer agent compared with other drugs. Tridecyne it another compound that was detected; it is a saturated aliphatic hydrocarbon compound, and it is used as an alternative pathway for treating pathogenic microbes due to antibacterial and antifungal properties (65). Dihydromyrcenol is identified as one of the compounds that has a biological effect, and it acts as an antibacterial, antioxidant, antiulcer, anti–anti-inflammatory, and anticancer (66).

Conclusion

In current study gave a high percentage for isolation of *Staphylococcus aureus* from skin infection, and

it appeared multi- drag multidrug-resistant to antibiotics. The Honey has potential biological effect and could be used as antibacterial agent against *Staphylococcus aureus*, the high value of antibacterial effect of the honey can be obtain from concentration 100% and effect was decrease when less dilution of honey, there are (11) important compound demonstrated in honey was analysis by GC-MS technique and all these compound was discovered has antibacterial effectiveness, this can support to applied honey for treatment infection of skin and assisted of wounds healing.

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