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Growth inhibition of *Pseudomonas aeruginosa* using products of some probiotic microorganisms and secondary metabolites of *Commiphora myrrha* extracts estimated by GC-MS technique

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Abstract

The search for new antibacterial agents is necessary due to the limited availability of effective treatments to treat bacterial infections. The current study aimed to study the utilization of the products of some probiotic microorganisms, such as *Lactobacillus acidophilus* and *Saccharomyces boulardii*, are cultured on solid and liquid media, as well as examining the biological activity of the secondary metabolites of the aqueous, alcoholic, and vinegar extracts of the *Commiphora myrrha* (Myrrh) plant, and honey on the growth of five clinical isolates of *Pseudomonas aeruginosa* isolated from wounds using the diffusion method with tablets. Some chemical tests were conducted to identify the effective compounds of the extracts using qualitative analysis of the chemical compounds in the plant. In addition to the high antioxidant effect, which was recorded at about 64.84, the results of the Gas Chromatography-Mass Spectrometry (GC-MS) is an analysis to identify different substances into compounds in aqueous extract and ethanolic. The results of aqueous, alcoholic, vinegar and honey extracts of *C. myrrha* showed that all studied extracts have the ability to inhibit *P. aeruginosa*, but to different extents. The aqueous extract gave better results than the alcoholic extracts, and vinegar. As for honey, it did not have a synergistic inhibitory effect when mixed with the myrrh plant extract. As for *L. acidophilus* had an inhibitory effect on growth in solid agar for all tested isolates, while the use of bacterial free- cell supernatant did not show any inhibitory activity against bacteria.

Keywords: Antioxidant effect, *Commiphora myrrha*, GC-MS technology, Probiotic microorganism, *Pseudomonas aeruginosa*.

Introduction

Pseudomonas aeruginosa, is one of the most important bacterial species that cause what is known as nosocomial infection in hospitals, as it can grow on the floor of hospital lobbies, operating rooms, surgical instruments, etc., and this bacterium also has the ability to survive in disinfectants and some types of sterilizers [1]. The infection with these bacteria is often localized or specific to a specific system, such as injuries to the urinary tract, wounds, ear, eye, and sometimes the respiratory system [2]. It causes especially wound infections, and the spread of these bacteria in burn treatment units in hospitals is still associated with a death rate of more than 60%[3].

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In the past years, a clear interest in the use of medicinal plants has emerged with the development of countries due to the entry of derivatives of these plants into the manufacture of medicines, which were found to be safe, and their use does not lead to the appearance of side effects[4]. The *Commiphora myrrha* (Myrrh) plant is one of the genera of the family resinous plants that are used in various medical fields. This plant is considered an anti-parasite, as it treats some worms such as *Fasciola* [5], as well as intestinal disorders, diarrhea, respiratory congestion, and skin infections [6], and is used as an anti-inflammatory plant [7]. It is an excellent medicine for mouth and throat problems and has a slightly bitter taste. It has antibacterial and antifungal effects against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* [8]. Honey has also been found to have a superior ability to sterilize and have antibacterial properties. It has been used successfully in disinfecting and dressing wounds [9]. The researchers found that honey may kill bacteria, and perhaps one day it will replace antibiotics for [10, 11]. Therapeutic organisms were also used in this study, where probiotics, or what are called symbiotic bacteria, which were known as live bacterial food supplements that benefit the host by improving the balance of the natural intestinal micro flora [12]. Lactic acid bacteria are the most common species in the therapeutic and nutritional fields, because they possess many characteristics that are rarely met in other organisms, such as their growth in the presence or absence of air, resistance to low pH, fast growth, taste, and distinct odor [13], in addition to the use of these bacteria, *Saccharomyces boulardii* was also used, which is considered one of the most active types of yeasts in the world and does not have any side effect on animal health. It competes with any harmful bacteria in the intestines and weakens them by producing digestive enzymes [14]. Therefore, the aim of current research is to investigate the biological effectiveness of Myrrh plant extracts, and honey compare them with the use of some therapeutic organisms products and use them as alternatives to antibiotics in the field of medicine industry or even as a substitute for preservatives in the field of the food industry.

Materials and Methods

Bacterial isolates

Pseudomonas aeruginosa isolates were obtained from clinical sources of burn injuries and were identified and activated on nutrient agar according to Shehab *et al.*[15]. As for the probiotic isolates, which include *L. acidophilus* and *S. boulardii* were obtained as identified isolates from the microbiology laboratory of the College of Science for Girls / University of Baghdad, and they were activated and cultured on MRS broth and Sabrouraud agar, respectively, according to Rezuqi *and Younis* [16], and then kept in the refrigerator until use.

Plant material

The Myrrh plant was gained in the form of gum from herbalists' shops in the local markets in the city of Baghdad, where it was ground and turned into a powder and kept in glassware in the shade until use.

Preparing the infusion (skin lotion)

Ten grams of the Myrrh resin were solved in 100 ml of sterilized distilled water and put in a water bath to boil slowly at 60 °C for 2 hours. Then it was filtered through we filter it with sterile filter papers, and the resulting filtrate was placed in a centrifuge for separation at a speed of 5000 rpm for 10 minutes. Then the resulting infusion was re-boiled until the solvent was reduced to one to four of the original volume of the solvent, then used or kept in the refrigerator at 4 °C until use [17].

Preparation of tinctures

Twenty grams of Myrrh resins were weighed per 100 ml. of ethanol in an opaque glass beaker, and the ratio of preparation was 5: 1 (any part of the herb to five parts of alcohol). The beaker was shaken well for (1-2) minutes, then kept in a cold and dark place for a period of 10-14 days, and shaken well every day. After the period ended, the liquid was poured into sterilized dark glass bottles and the herb was compressed in several layers of

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gauze until the tincture drip stopped and kept airtight, and preserved until use. The vinegar tincture was prepared as mentioned above [18].

Qualitative chemical detection of some active groups of Myrrh extracts

Some chemical tests were conducted to investigate active groups of Myrrh extracts, where alkaloids, saponins, phenols, tannins, flavonoids, resins, glycosides, coumarins, and pH were detected according to the method mentioned by Al-Hayanni *et al.* [19].

DPPH radical scavenging activity of Myrrh resins

Forty milligrams of DPPH were dissolved in 100 ml of methanol, and the concentration of DPPH was 400ug/ ml. To prepare the standard solution (vitamin C) and sample, 0.5 g of vitamin C was mixed with 100 ml of methanol and distilled water. The concentration of the standard solution was 5000 ppm and, the other concentrations were prepared (30, 60, 120, 250, 500 ppm) from vitamin C and sample (30, 60, 120, 250, 500 ppm). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 517 nm using a spectrophotometer (UV-VIS Shimadzu). The IC_{50} value of the sample was calculated using a Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity[20].

The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A1 / A0 × 100.

Where A0 was the Absorbance of the blank and A1 was the Absorbance in the presence of the test sample.

Gas Chromatography-Mass Spectrometry (GC-MS) for identification of Unknown compounds.

The active compounds of *C. myrrha* resins were identified using a Gas Chromatography Mass Spectrometer GC- MS (5977E) USA, by comparing the resulting absorbance of the unknown compounds with stored and known components in a library of the National Institute of Standards and Technology (NIST).

Preparation of bacterial and yeast suspensions

Four to five separate bacterial or yeast colonies were selected and transferred to a test tube containing (10) ml of MRS broth for *L. acidophilus*, and Sabrouraud broth for *S. boulardii*, respectively, and incubated at 37 °C. For a period of (5-6) hours until turbidity appears. This turbidity was compared with a standard suspension, which is a McFarland tube 0.5, to obtain a microbial suspension with a concentration of 1.5 x 10^8 cells/ ml [21].

Effect of alcohol, vinegar tinctures, and aqueous infusion of *C. myrrha* on the growth of *Pseudomonas aeruginosa*

The effect of aqueous, ethanol, and vinegar extracts of Myrrh on the growth of *P. aeruginosa* bacteria was studied, which was prepared using well diffusion method, where 10 ml of nutrient broth medium was inoculated for all studied bacterial isolates and incubated at 37 °C for 24 hours, then drilling was done in the solid agar using a cork puncture measuring 6 mm, 3 holes were made and 0.1 ml of each extract was placed in them separately, and each extract was tested for the five isolates and incubated at 37 °C for 24 hours. The diameter of the inhibition zone was measured in millimeters minus the diameter of the hole. Vinegar and ethanol were tested alone as controls on the growth of *P. aeruginosa* bacteria using the same method used above [22].

The inhibitory effect of *Lactobacillus acidophilus* and *Saccharomyces boulardii* on solid media (disc diffusion method)

Lactobacillus acidophilus and *S. boulardii* were inoculated in MRS agar and Sabrouraud agar, and then the plates were incubated under suitable conditions at 37 °C for 48 hours. After incubation, the tubes were centrifuged for five minutes at 4000 rpm, and then the supernatant was taken for study. Then the sterilized discs were inoculated from the culture with a diameter of 5 mm from each medium 3 discs were placed on each plate for all five isolates of bacteria) were placed on the surface of the nutrient agar which was spread with *P. aeruginosa* for all isolates, then the plates were incubated at 37 °C for 24 hours, after which the diameter of the inhibition zone around the discs was measured in millimeter [23]. In addition, the effectiveness of *L. acidophilus* and *S. boulardii* filtrates (their broth growth) were tested against *Pseudomonas aeruginosa* isolates, according to the method of Ronald [24, 25], using the disc diffusion method.

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Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. The least significant difference –LSD test (Analysis of Variation-ANOVA- Completely Randomized Design-CRD in rows and columns) was used to significance compare between means in this study [26].

Results and Discussion

The results of the qualitative chemical detection for the effective groups of aqueous, ethanol and vinegar Myrrh resin extracts in Table 1, show that is contain alkaloids, saponins, phenols, tannins, flavonoids, glycosides, coumarins, resins and pH varied in their positivity according to the different types of solvents, its polarity, and its pH. The above-mentioned compounds are secondary metabolites, which have a defensive importance for plants against microorganisms, as well as benefited humans in the various fields of food and medicine [27].

Table 1. Chemical composition of some active compounds of Myrrh extracts.

Active compounds	Type of extract		
	Aqueous extract (skin lotion)	Myrrh ethanol	Myrrh Vinegar extract
Alkaloids	-	-	+
Saponins	+	++	+
Phenols	–	-	+
Tannins	–	-	+
Flavonoids	-	+	+
Resins	+++	++	-
Glycosides	-	-	+
Coumarins	-	+	-
PH	5.42	5.16	4.32

The DPPH radical-scavenging activity % of *C. myrrha* resin methanol extract at a concentration of 400 µg/ml is shown in Fig. 1. The LC_{50} value of the sample, which is the concentration of sample required to inhibit 50 % of the DPPH-free radical, was 64.84 %. The standard scavenging activity of Vitamin C exhibited much lower than the scavenging activity of Myrrh extract.

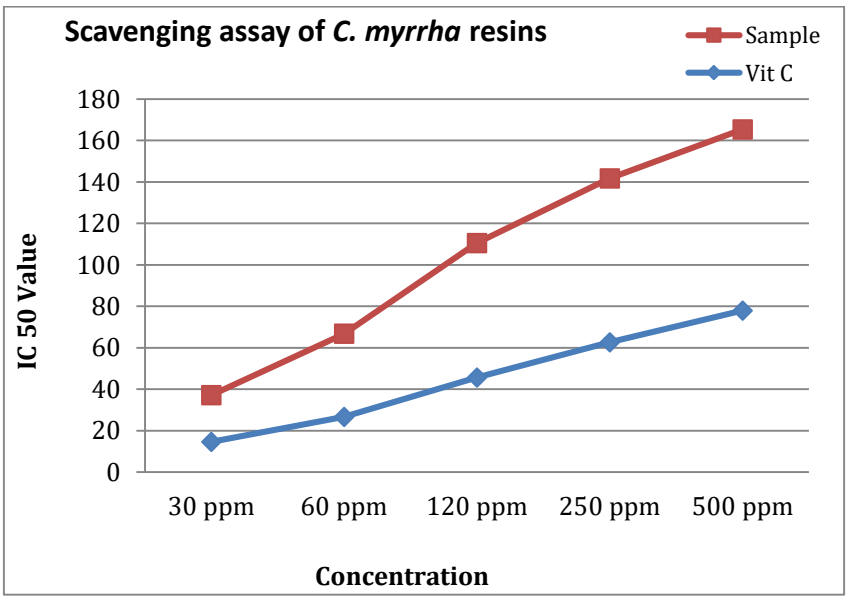

Figure 1. DPPH free radical scavenging assay of *C. myrrha* resins.

Fig. 2. and Table 2. show the chemical compounds present in the aqueous extract of C. myrrha resins, which were detected by gas chromatography using mass spectrometry, as the detection showed the presence of 25 compounds, and the highest peak area of the Myrrh aqueous extract was 22.37 % in

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the minute 34.0 for the compound alpha.-Amyrin, trimethylsilyl ether, D-Norandrostan-16-ol, acetate, alpha.,16.beta.), 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methyl ethyl lidene)-(8S-cis) and the lowest peak area was 0.46% in the minute 27.2 for the compound 1,2-Hydrazinedicarboxylic acid, diethyl ester, Hydroperoxide, heptyl, 3-Butyn-1-ol. Fig. 3 and Table 3. displayed the chemical compounds exist in the ethanolic extract of C. myrrha resins, as the detection showed the occurrence of 30 compounds, and the highest peak area of the Myrrh extract was 24.34 % in the minute 34.13 for the compound alpha. Alpha -Amyrin, trimethylsilyl ether, D-Norandrostan-16-ol, acetate, (5.alpha.,16.beta.), 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis) and the lowest peak area was 0.43% in the minute 34.6 for the compound Acetonitrile. The compounds varied in their appearance time, and there were compounds that appeared repeatedly in different periods of time.

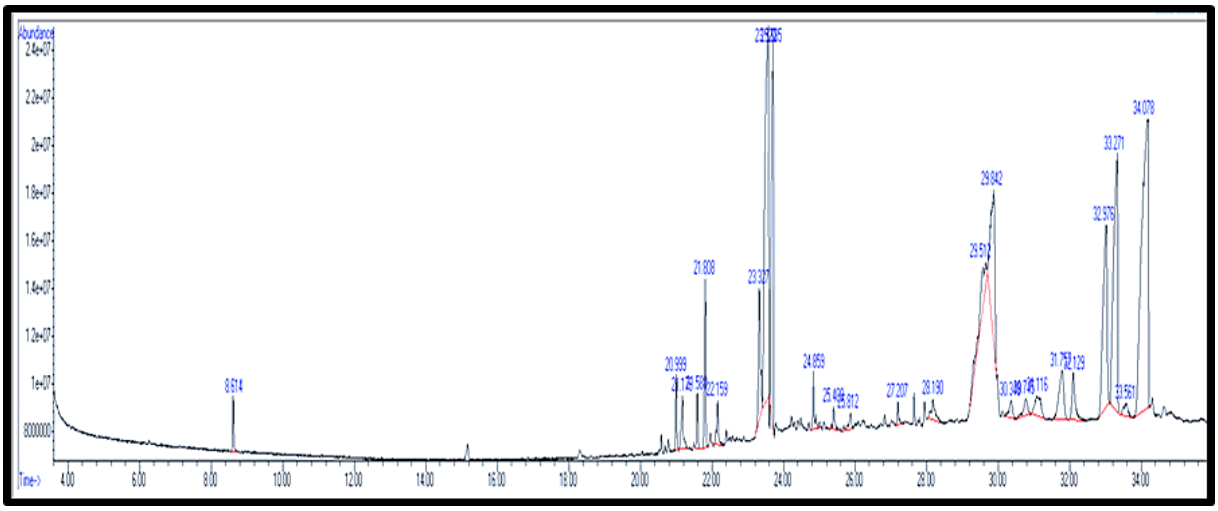

Figure 2. Chromatogram of *Commiphora myrrha* chemical compounds of aqueous extract identified by GC-MS.

Table 2. *Commiphora myrrh*a chemical compounds of aqueous extract

Peak no.	Compound name	RT	Area%
1	Acetic acid, octyl ester	8.616	**0.72**
2	1,5,9-Cyclotetradecatriene, 1,5,9-trimethyl-12-(1-methylethenyl) , Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)] Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]	21.001	**0.95**
3	n-Hexadecanoic acid Pentadecanoic acid	21.174	**1.21**
4	3-Cyclohexene-1-methanol, .alpha., .alpha.,4-trimethyl-, acetate , 3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl) , 3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, propanoate	21.590	**0.74**
5	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)] Bicyclo[2.2.1]heptane, 2-cycloprop ylidene-1,7,7-trimethyl (-)-delta.-Panasinsine	21.806	**2.38**
6	Caryophyllene Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	22.161	**0.84**
7	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)] Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	23.330	**3.36**

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8	3-Cyclopentylpropionic acid, butyn-2-yl ester 4-(2-Chloro-benzyloxy)-3,5-diiodo benzaldehyde 3-Bromo-1,2-dichloro-1-phenylpropane	23.520	**15.57**
9	Propanoic acid, 2-chloro-, 1-methylbutyl ester 1-Butoxy-2,4-dimethyl-2-pentene Octane, 2,3,6,7-tetramethyl	23.685	**9.70**
10	Carbamic acid, (2-chloroethylidene)bis-, diethyl ester 1,2-Hydrazinedicarboxylic acid, diethyl ester Oxirane, (ethoxymethyl)	24.862	**1.16**
11	Diethyl sulfate , 3-Butyn-1-ol	25.407	**0.50**
12	Hydroperoxide, heptyl Carbamic acid, (2-chloroethylidene)bis-, diethyl ester 1,2-Hydrazinedicarboxylic acid, diethyl ester	25.814	**0.58**
13	1,2-Hydrazinedicarboxylic acid, diethyl ester , Hydroperoxide, heptyl , 3-Butyn-1-ol	27.207	**0.46**
14	Spirohexan-4-one, 5-chloro-6,6-dimethyl 2-Chloro-1,1,2-trifluoroethyl ethyl ether 4(1H)-Pyrimidinone, 2-(ethylthio)	28.194	**0.95**
15	Tetrahydropyrrole-2,5-dione, 1,3-dimethyl-3-[2-nitro-5-methoxyphenyl] 2-(2,5-Dimethoxyphenyl)cyclohexenone Ethyl 6-methyl-4-oxo-4H-chromene carboxylate	29.510	**3.35**
16	2-(2,5-Dimethoxyphenyl)cyclohexenone Tetrahydropyrrole-2,5-dione, 1,3-dimethyl-3-[2-nitro-5-methoxyphenyl] N-(3,5-Di-t-butylphenyl)acetamide	29.838	**6.95**
17	.beta.-Amyrin Pyrene, hexadecahydro	30.349	**0.72**
18	3-Butyn-1-ol	30.747	**0.88**
19	.alpha.-Amyrin, .beta.-Amyrin , Indeno[2,1-b]chromene	31.119	**1.52**
20	.alpha.-Amyrin , .alpha.-Amyrin, trimethylsilyl ether beta.-Amyrin	31.760	**2.70**
21	3-Butyn-1-ol	32.132	**1.66**
22	3,5,5'-Trihydroxy-3'-methoxy-2,2'-binaphthalene-1,1',4,4'-tetrone , Perylene, 3-(2-naphthalenylmethyl) Silane, dimethyl(3-ethylphenoxy)tetradecyloxy	32.980	**8.28**
23	Pyrrolo[2,3-b]indole, 1,2,3,3a,8,8 a-hexahydro-5-methoxy-3a,8-dimethyl , Anthracene, 9-(2-propenyl) .beta.-Amyrin trimethylsilyl ether	33.275	**11.74**
24	Hydroperoxide, heptyl, 3-Butyn-1-ol , 1,2-Hydrazinedicarboxylic acid, diethyl ester	33.560	**0.70**
25	.alpha.-Amyrin, trimethylsilyl ether D-Norandrostan-16-ol, acetate, alpha.,16.beta.) 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethy lidene)-, (8S-cis)	34.080	**22.37**

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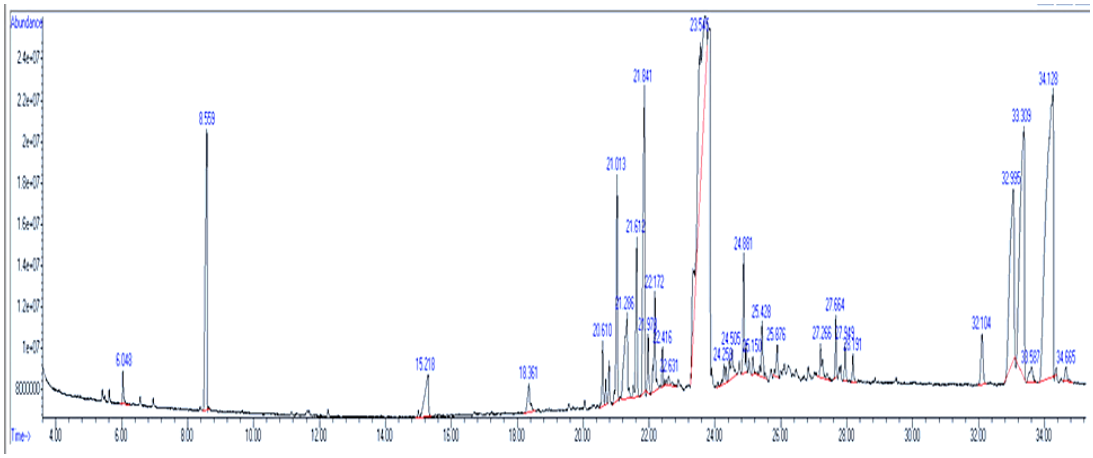

Figure 3. Chromatogram of *Commiphora myrrha* chemical compounds of ethanolic extract identified by GC-MS.

Table 3. *Commiphora myrrh*a chemical compounds of ethanolic extract.

Peak no.	Compound name	RT	Area%
1	Formic acid, octyl ester, Octanol	6.045	**0.45**
2	Acetic acid, octyl ester	8.564	**5.73**
3	Dodecanoic acid	15.220	**1.90**
4	Tetradecanoic acid	18.362	**0.87**
5	Acetonitrile	20.612	**1.22**
6	Cyclotetradecatriene , trimethyl12-(1-methylethenyl)-Cyclohexane, 1-ethenyl-1-methyl-2 ,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)] Cyclohexane, 1-ethenyl-1-methyl-2	21.010	**3.50**
7	n-Hexadecanoic acid , Pentadecanoic acid	21.287	**3.29**
8	3-Cyclohexene-1-methanol, .alpha., .alpha.,4-trimethyl-, acetate 3-Cyclohexene-1-methanol,.alpha.,.alpha.,4-trimethyl-, propanoate Cyclohexanol, 1-methyl-4-(1-methy ethylidene)-l	21.616	**2.82**
9	Bicyclo[9.3.1]pentadeca-3,7-dien-2-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)] Bicyclo[2.2.1]heptane, 2-cycloprop ylidene-1,7,7-trimethyl 17-Norkaur-15-ene, 13-methyl-, beta.,13.beta.)	21.841	**6.59**
10	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl , Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl , Caryophyllene	21.980	**0.77**
11	Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl - Guaia-1(10),11-diene , 11,11-Dimethyl-spiro[2,9]dodeca-3	22.170	**1.90**
12	1H-1,4-Diazepine, hexahydro-1- yridinyl) Silane, tributylchloro , Benzeneacetonitrile, 3,4-dimethoxy	22.412	**0.53**
13	Acetonitrile, bromo-	22.629	**0.33**
14	3-[4-Methyl-1-piperazinyl]-6-[2-na phthalenyl]-1,2,4,5-tetrazine , 3-Cyclopentylpropionic acid, but yn-2-yl ester Cyclopentanol, 1,2-dimethyl- ethylethenyl)-, [1R-(1.alpha.,2.be ta.,3.alpha.)]	23.546	**13.11**
15	1,2-Cyclobutanedicarboxylic acid, 3-methyl-, dimethyl ester Spirohexan-4-one, 5,5-dimethyl	24.256	**0.67**
16	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester 9-Octadecenoic acid, methyl ester,(E)	24.507	**1.09**
17	5-Hydroxymethyl-1,3,3-trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclopentanol , 2,6,10,14-Hexadecatetraen-1-ol, 7,11,15-tetramethyl-, acetate, (E,E,E) Cyclopentanemethanol, 1-hydroxy-.alpha.,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)	24.879	**2.12**
18	2-Bromopropionyl bromide , 3-Bromopropionyl chloride , 1,3-Dioxane,	25.147	**0.29**

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	5-bromo-5-nitro-		
	2,6,10,14-Hexadecatetraen-1-ol, 3, 7,11,15-tetramethyl-, acetate, (E,E,E)		
19	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	25.424	**1.15**
	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)		
20	2,2-Dimethyl-1-(3-oxo-but-1-enyl)-cyclopentanecarboxaldehyde	25.874	**0.67**
21	Furazan, dimethyl 3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl	27.268	**1.00**
	Succinic acid, cyclohex-2-enylmethyl nonyl ester		
22	Fumaric acid, cyclohex-3-enylmethyl heptadecyl ester Fumaric acid, cyclohex-3-enylmethyl dodecyl ester	27.666	**0.81**
	Kauran-18-oic acid, 16-hydroxy ,4.alpha.)		
23	3-Cyclohexene-1-ethanol, .beta.,4-dimethyl	27.952	**0.54**
	Tetracyclo[5.2.1.0(2,6).0(3,5)]decane, 4,4-dimethyl		
24	No matches found	28.194	**0.38**
	Silane, dimethyl(2-isopropylphenoxy)tridecyloxy		
25	3,5,5'-Trihydroxy-3'-methoxy-2,2'-binaphthalene-1,1',4,4'-tetrone , Perylene, 3-(2-naphthalenylmethyl)	32.106	**1.37**
26	3,5,5'-Trihydroxy-3'-methoxy-2,2'-binaphthalene-1,1',4,4'-tetrone ,Perylene, 3-(2-naphthalenylmethyl)	32.998	**8.86**
	Silane, dimethyl(3-ethylphenoxy)tetradecyloxy		
	Pyrrolo[2,3-b]indole, a-hexahydro-5-methoxy-3a,8-dimethyl-,.beta.-		
27	Amyrin trimethylsilyl ether , Naphthalene, 2-(phenylmethyl)	33.309	**12.57**
28	No matches found	33.586	**0.69**
	alpha.-Amyrin, trimethylsilyl ether		
29	D-Norandrostan-16-ol, acetate, (5.alpha.,16.beta.) 5(1H)-Azulenone,2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)	34.132	**24.34**
30	Acetonitrile, bromo-	34.668	**0.43**

The results in table 4. show that aqueous and alcoholic extracts of Myrrh plant and vinegar only had a strong inhibitory effect on the growth of *P. aeruginosa*, whereas alcohol extract had a greater inhibitory effect than other extracts, where the largest inhibition zone was 37 mm for isolate No 1, while the least inhibition zone was of isolate No 2 reached to 19 mm., and vinegar extract of myrrh plant showed the highest inhibition in isolate No 1 (26 mm) and the lowest inhibition was in isolate No 3, where the inhibition zone reached 15 mm, while the highest inhibition zone was in isolate No 1 (35 mm) and the least inhibition zone was in isolate No 4 (25 mm). Vinegar and alcohol alone had a significant inhibitory effect on the growth of bacteria under study, where the highest result was in alcohol only against isolate No 2 (inhibition diameter 33 mm) and the lowest diameter was in isolate No 4, where the diameter was 19 mm while it did not affect the fifth isolate, In case of using vinegar only, the highest inhibition diameter was in isolate No 1 (31 mm).

Table 4. Effect of boiled aqueous, ethanol and vinegar extracts of Myrrh and effect of ethanol and vinegar alone on the growth of *Pseudomonas aeruginosa* measured by millimeter.

Isolate code no	Boiling water extract of Myrrh	Ethanolic extract of Myrrh	Vinegar extract of Myrrh	Ethanol only (+ve C)	Vinegar only (+ve C)	LSD value
No 1	37	35	26	26	31	**4.911 ***
No 2	28	19	17	33	18	**5.407 ***
No 3	34	39	15	21	18	**7.229 ***
No 4	25	21	16	19	25	**5.024 ***
No 5	28	27	17	0	0	**5.871 ***
LSD value	5.692 *	6.027 *	5.403 *	5.977 *	5.318 *	**---**

*** (P≤0.05).**

The results in Table 5, show that the effect of honey and honey wax with Myrrh plant had a higher inhibitory effect than the use of honey and its wax alone, where the highest inhibition diameter of

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honey with Myrrh plant on isolate No 5 (35 mm) and the lowest diameter was for isolates No1 and No 3 was 17 mm, on the other side the highest inhibition diameter in honey (wax) with Myrrh plant was on isolate No 3 reached 17 mm and the lowest diameter was 7 mm in isolate No 4 and did not affect the isolate No 5. The effect of honey only and wax only was only in isolate No 2, where it reached 12 and 7.6 mm respectively.

Table 5. The antibacterial effect of honey wax and honey alone on the growth of bacteria, and the synergistic effect of honey (wax and honey) with Myrrh on the growth of *P. aeruginosa* isolates (as inhibition zones in mm)

Isolate code no.	Honey wax only	Honey only	Honey with Myrrh	Honey wax with Myrrh	LSD value
No 1	0	0	17	9	**4.076 ***
No 2	8	12	20	16	**4.823 ***
No 3	0	0	17	17	**4.905 ***
No 4	0	0	24	7	**5.169 ***
No 5	0	0	35	0	**4.752 ***
LSD value	3.156 *	3.866 *	5.704 *	4.945 *	---

*** (P≤0.05).**

Results in Table 6, showed that *Lactobacillus acidophilus* cultivated on a solid medium had a more inhibitory effect on the growth of *P. aeruginosa* than filtrate of this bacterium. The highest inhibition was on isolate No 2, (19 mm), while the least inhibition diameter was on isolate No 3, (3 mm). Filtrate of *L. acidophilus* did not show any inhibitory effect except on isolate No 5, where the inhibition diameter reached 9 mm. As for the yeast of *Saccharomyces*, it did not show any inhibitory effect on the growth of *Pseudomonas i*solates, whether in the case of solid or suspended agar, except for isolate No 1, where the inhibition diameter reached 3 mm.

Table 6. The antibacterial effect of *L. acidophilus and S. boulardii* cultured on the solid medium and the supernatant on the growth of *Pseudomonas aeruginosa* measured by millimeter.

Isolate code no.	Treatment				LSD value
	Growth of *L. acidophilus* by disc method	**Supernatant of *L. acidophilus* by disc method**	**Growth of *S. boulardii* by disc method**	**Supernatant of *S. boulardii* by disc method**	
No 1	17	0	0	0	**4.528 ***
No 2	19	0	0	0	**4.822 ***
No 3	3	0	0	0	**3.00 NS**
No 4	9	0	0	0	**4.785 ***
No 5	4	9	0	0	**4.271 ***
LSD value	4.027 *	3.377 *	0.00 NS	0.00 NS	---

*** (P≤0.05).**

It follows from the previous results that all the studied extracts had the ability to inhibit *P. auroginosa,* but to a varying degree, due to the different extraction methods used and the difference in the polarity of the solvent, which led to the different content of extracts from the active groups [22].The highest DPPH radical scavenging activity of methanol extract may be referred to the high concentration of sesquiterpenoids, diterpenes, triterpenes, and sterols in Myrrh which might be the electron donors and henceforward can react with free radicals to convert into more stable products and terminate radical chain reactions [28]. The active compounds differed in the extracts. This may be due to the difference in the current study with previous studies in the extraction period, extraction methods, the used part of the plant, the environmental factors, the time of plant collection,

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and the growing season, all these reasons make differences in the chemical compounds of plants. The presence of many active compounds containing hydroxyl groups and a group of Carbonyl double bonds that are known to possess diverse biological activities such as antioxidants, antibiotics, and anti-inflammatories in addition to their chemo-preventive importance [20]. The difference in the anti-microbiological effectiveness of plant extracts depends on the type of plant and microorganism [23]. It has been observed from tables 3 and 4, that the effect of infusion of the Myrrh plant is highly inhibitive compared to the rest of the extracts, due to the fact that this plant has characteristics and properties It is important in recent research and studies that this effectiveness is due to Terpenes, especially (furanoses and quiterpenes) and active compounds that are mainly found in the form of oils, in addition to the presence of gum by 30-60% polysaccharides and resin by 25-40% and volatile oils by 3-8% include (herabolin, eugenol, and many furanoscoterpenes) [29]. It was found that the results of some researchers [21], were less than our results of the aqueous and alcoholic extracts that we have done, as well as it was also found that the aqueous and alcoholic extract of the Myrrh resins was effectively inhibited the growth of *P. aeruginosa* isolates, and this effectiveness was due to the effective compounds found in the bitter plant, where it was found that Alkaloids and Saponins present in the plant are due to its inhibitory effectiveness against bacteria. Inhibition of the growth of bacteria, as it was highly effective inhibitory when mixed with the plant of myrrh where the relationship between them was called synergism[30], but the mechanism of this interaction between medicinal plants and honey is still unclear, and this mixture is used to treat ulcers that affect the foot in diabetics. It has been found that this effectiveness of honey is due to the fact that the high concentrations of honey led to inhibition of bacterial growth, and this is due to the high concentration of sugars, which may lead to the process of plasmolysis, which is the process of withdrawing water from the body of bacteria. The other important reason, as confirmed by many studies, is the presence of antibacterial substances in natural honey that help inhibit the growth of germs [31]. In addition to the above, the high acidity of honey may be another reason, studies have proven that the high acidity of honey has a role in inhibiting the growth of germs in addition to the previous factors [32] and this acidity in honey is produced by the enzymes secreted by bee workers on nectar when converted into mature honey such as the enzyme Glucosoxidase in addition to the action of other enzymes during the ripening process, in addition to its low PH osmosis. [33], Data in Table 5 show that lactic acid bacteria cultivated on solid agar had an inhibitory effect on the growth of *Pseudomonas aeruginosa* bacteria has been found that lactic acid bacteria have an inhibitory effect for the Gram negative bacteria and this was observed through the report of Rezuqi and Younis [16] also on *E.coli*. It has been found that organic acids and lactic hydrogen peroxide, acid, and bacteriocins are responsible for the inhibitory ability of lactic acid bacteria, and the effectiveness of lactic acid is due to the no separated lactic acid molecules and lowering the PH level helps in inhibition [34,35]. While it was noted through the same table that the effect of lactic acid bacteria in a suspended form, as well as the yeast of *Saccharomyces boulardii* cultivated on the agar as well as broth, did not limit the inhibitory effect on the growth of *P. aeruginosa* and this may be due to the effectiveness of the bacterial isolates, resistance, and virulence, where *P. aeruginosa* produces many virulence factors that enable it to break down tissues and invade the bloodstream, and the most important of these factors is the enzyme hemolysin or the state of the blood. , the enzyme elastase that breaks down the elastin protein, collagen, a number of immunoglobulins, the enzyme urease that breaks down urea, and other enzymes such as exogenous enzyme (S) and (U) as well as many bacterial toxins such as exotoxin (A) and others [33].

Conclusion

The current study gives important results not only for the discovery of new compounds for secondary *Commiphora myrrha* metabolites using GC-MS technology but also for the exact concentrations of these organic and inorganic compounds. This analysis can support the importance of the Myrrh plant for use as a treatment, especially since the plant contains the vital basic elements necessary for healing wounds and inhibiting some types of bacteria such *as P. aeruginosa*. The study also revealed the use of secondary metabolites of *Lactobacillus acidophilus* through growth on solid media, which gave effective growth inhibition results on solid media for all tested isolates.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contribution Statement

Z.H.S. Planned the research work and performed the research work, performed the experiments and contributed to the writing, and data analysis of the paper and T.H.M. also performed the experiments, contributed to the writing, publishing the paper as correspondence author. A.A.T. Contributed to the writing of the paper and data collection.

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تثبيط نمو الزوائف الزنجارية باستخدام منتجات بعض الاحياء العلاجية ونواتج الايض الثانوي لمستخلصات نبات المر المقدرة بتقنية كروموتوغرافيا الغاز

زينه هاشم شهاب، طيبة هاشم محمد، اروى عبد الكريم توفيق

قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة

ان محدودية توفر العلاجات الفعالة لمعالجة البكتريا، والبحث عن عوامل جديدة مضادة للبكتيريا أمر ضروري. لذلك هدفت الدراسة الحالية الى استخدام تأثيرنواتج بعض الاحياء العلاجية مثل *Lactobacillus acidophilus* و *Saccharomyces boulardii* المنماة في الأوساط الصلبة والسائلة، وكذلك فحص النشاط الحيوي لنواتج الايض الثانوي للمستخلصات المائية والكحولية والخل لنبات *Commiphora myrrha* (المر) على نمو خمس عزلات من الزوائف الزنجارية المعزولة من الجروح باستخدام طريقة الانتشار بالاقراص. اظهرت بعض الاختبارات الكيميائية على وجود عدد من المركبات الفعالة للمستخلصات النباتية باستخدام التحليل النوعي للمركبات الكيميائية في النبات، اضافة الى التأثير العالي المضاد للأكسدة حيث سجل حوالي 64.84، بينما توصلت نتائج تقنية كروموتوغرافيا الغاز مركبات جديدة مختلفة في المستخلص المائي و الإيثانولي. كما أظهرت نتائج المستخلصات المائية والكحولية والخل والعسل لنبات *C. myrrha* أن جميع المستخلصات المدروسة لديها القدرة على تثبيط الزوائف الزنجارية ولكن في نطاقات مختلفة. أعطى المستخلص المائي نتائج أعلى من المستخلصات الكحولية والخل وكذلك العسل، أما العسل فلم يكن له تأثير تثبيط تآزري عند مزجه مع نبات المر. اما العصيات اللبنية اعطت تأثير مثبط للنمو في الأجار الصلب لجميع العزلات المختبرة ، بينما لم يظهر استخدام الراشح البكتيري أي نشاط مثبط ضد البكتيريا .

الكلمات المفتاحية: الفعالية المضادة للاكسدة، *Commiphora myrrha*، تقنية كروموتوغرافيا الغاز، الاحياء العلاجية، الزوائف الزنجارية.