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ANTIBACTERIAL PROPERTIES AND PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT OF OLEO-GUM RESINS OF *COMMIPHORA MYRRHA* AND *COMMIPHORA MOLMOL*

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ABSTRACT

The present study was performed to test the effectiveness of the aqueous extracts of *Commiphora myrrha and Commiphora molmol* in inhibition of four types of pathogenic bacteria; *Micrococcus luteus, Neisseria siccca, Proteus mirabilis* and *Pseudomonas aeruginosa*. The results showed that the aqueous extracts of *C. myrrha and C. molmol* have an inhibitive effect on the growth of each bacterium tested. The inhibition of bacterial growth decreased as the storage period of the myrrha was increased. *Commiphora myrrha* lost its inhibitive effect on *Proteus mirabilis* and *Micrococcus luteus* when myrrha was stored for one month, sex months and one year. The aqueous extract of *Commiphora molmol* lost its effect against *Proteus mirabilis* after one year of storage. *C. molmol* was seen when used as 50% of the concentration and stored only for a month as the inhibitive area decreased to 2.47 cm^2 for *Micrococcus luteus*, 2.43 cm^2 for *Neisseria sicca*, 2.17 cm^2 for *Pseudomonas aeruginosa* and 1.78 cm^2 for *Proteus mirabilis*. Chemical analysis of myrrha showed that it contains three components, 2-fluorodiphenylmethane, Tribenzo-1,2,3,4,5,6anthracene and 2-bromo-1-(4-bromophenyl)-Ethanone, known for their microbial inhibitive effect. In addition, antimicrobial activities of 12 pharmaceutical bacterial antibiotics were tested against the four bacterial strains used in the experiment. It was found that *Micrococcus luteus* is the most resistant, as it was only inhibited by five of the 12 antibiotics tested followed by *Proteus mirabilis* that was inhibited by six antibiotics. The growth of *Pseudomonas aeruginosa* was inhibited by eight antibiotics and *Neisseria sicca* was the one most sensitive to the common antibiotics as it was inhibited by nine antibiotics.

Keyworeds: *Micrococcus luteus, Neisseria sicca, Proteus mirabilis, Pseudomonas aeruginosa, Commiphora molmol, Commiphora myrrha,* inhibition zone.

INTRODUCTION

Antimicrobial drugs have proved effectiveness in control of bacterial infections but since pathogens evolve, and develop resistance there is a continous search for antimicrobial agents present in the plants (Cowan, 1999). Many studies have been carried out on natural substances for their antifungal/antibacterial activities and their effects compared to antibiotics present in the market (Sakagami *et al*., 2001; Velickovic *et al*., 2003).

Natural antibiotics' aqueous extract is easily obtained and they have little side effects compared to synthetic antibiotics (Adel and Mahasneh, 1999; DeBoer *et al*., 2005). *Commiphora myrrha* and *Commiphora molmol* belong to Bruseraceae family and are commonly nown as "Myrrh". Myrrh is one of the important medical plant. The resin of the plant is used in the treatment of wounds, intestinal disorders, diarrhea, coughing, chest pain (Ghazanfar, 1994) and gingivitis (Serfaty and Itid, 1988).

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Rahman *et al*. (2008) reported that resin of *C.molmol* is effective against many strains of *Staphylococcus aureus*. We find that the antimicrobial components extracted from the plants hinder the growth of the bacteria through mechanisms different from those used currently by antibacterial agents and they may have great remedial value in resisting the strains of germs (Harborne, 1998).

The minimum inhibitory concentration (MIC) of the alcoholic extract of the myrrh that affects the tested strains of *S. aureus* bacteria ranges from 31.25 to 250mg/ml (Abdullah *et al*., 2009). Al Ahmadi (2006) reported that the *Commiphora* resins oils are rich with vuoranossiscottrbin and a total of 20 different compounds have been identified from this genus. Vuoranossiscottrbin separated compounds or *Commiphora* resin extracts showed antibacterial, antifungal as well as anesthetic properties. This study aimed at finding natural plant-sources that can inhibit the growth of *Micrococcus luteus, Neisseria sicca, Poteus mirabilis* and *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Plant samples

The plant-samples, *Commiphora myrrha* and *Commiphora molmol*, were collected from retail-shops in Dammam city. The samples bought were one month, sex months and a year old i.e. they were in the shop for that period of time in room temperature. The bought samples were kept at 4°C until tested.

Microbial isolates source

Four bacterial isolates, *Micrococcus luteus, Neisseria siccca, Proteus mirabilis* and *Pseudomonas aeruginosa* were obtained from Department of Microbiology, Dammam University. The microbial samples were kept at 4°C until the test.

Preparing the extracts

Aqueous extract

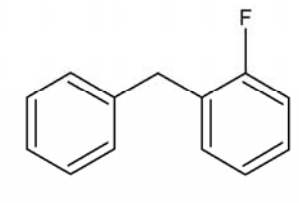
1, 2 and 5g powder of *C. myrrha and C. molmol* was dissolved in 10ml of sterilized distilled water. They were soaked at room temperature for 24hours, then filtered using layers of gauze (Boyraz and Ozcan, 2005).

Media preparation

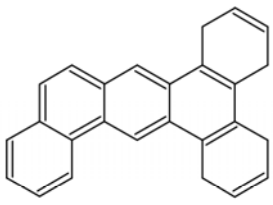
Nutrient Agar was prepared by dissolving 5g of meat extract, 2gm of yeast extract, 5gm of peptone extract, 5gm of sodium chloride extract and 15gm Agar in one liter of

Table 1. Inhibition zone (mm) of Myrrh extracts at various concentration on four microorganisms.

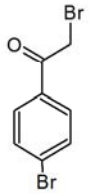
Type of myrrh	Storage period	Conc. of myrrh	Diameter of inhibition zone (mm)				Mean	
			Micrococcus luteus	*Neisseria siccca*	*Pseudomonas aeruginosa*	*Proteus mirabilis*		
C. myrrha	month	10	.68	1.66	1.17	0	.89	1.55
		30	1.59	2.05	2.08	.59	1.58	
		50	2.47	2.43	2.18	1.68	2.19	
	6month	10	0	1.08	1.07	.55	.68	1.10
		30	.78	1.32	1.09	1.65	1.21	
		50	.8	1.59	1.7	1.59	1.42	
	12month	10	0	1.25	.975	0	0	.41
		30	0	1.4	.98	0	.60	
		50	0	1.45	1.1	0	.64	
C. molmol	month	10	.53	.76	1.35	.67	.67	1.53
		30	1.48	2.28	1.93	1.81	1.89	
		50	1.69	2.45	1.98	1.99	2.03	
	6month	10	0	1.33	1.39	0	0	1.11
		30	.78	1.78	1.52	1.45	1.385	
		50	2.08	2.05	2.03	1.54	1.93	
	12month	10	0	.95	1.03	0	0	.76
		30	1.09	1.43	1.18	0	.930	
		50	2.35	1.68	1.34	0	1.34	
Mean			.91	1.61	1.45	.75		
# L.S.D.			.003	.019	.062	.003		



2-fluorodiphenylmethane



Tribenzo-1,2,3,4,5,6anthracene



2-bromo-1-(4-bromophenyl)-Ethanone

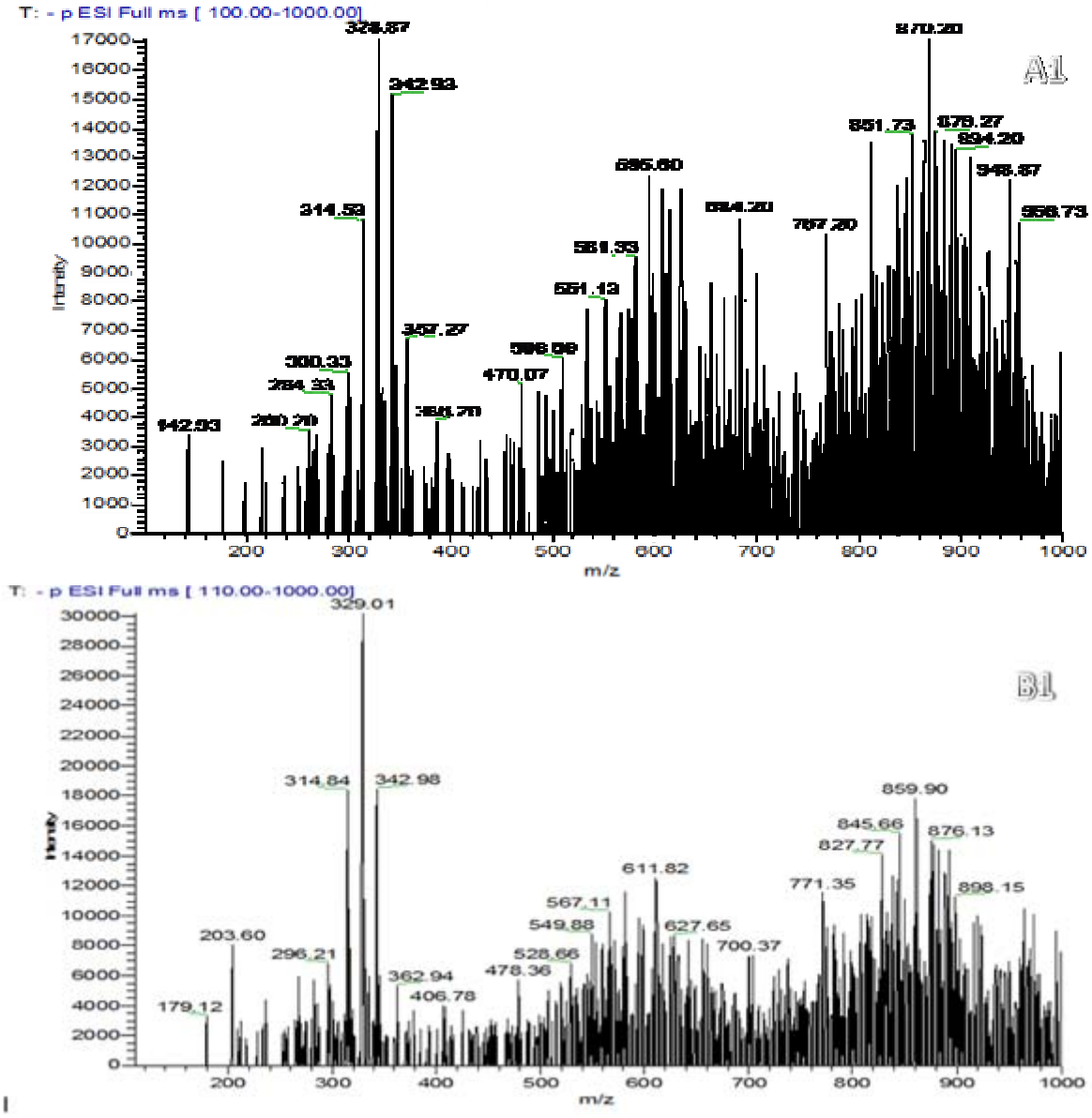


Fig. 1. Chromatograms of A1= Myrrh (*C. myrrha)* and B1=Myrrh (*C. molmol)* by mass spectrum after one month of storage.

water. Then transferred 250ml into flasks and autoclaved at 121°C for 15minutes.

Testing the effectiveness of aqueous extracts

The agar well diffusion method was used (Perez *et al*., 1990). 1ml of the Microbial inoculum was added in a sterile plastic petri dish and then 10ml of the medium was poured and left to harden. 1cm^2 holes were made and the extract was filled in holes, then incubated at temperature 23-30°C for 48hours. The results were recorded by calculating the inhibited area.

Detecting the composition of the chemical materials of Myrrha resin aqueous extract

Chemical composition analysis of myrrha was performed using mass spectrum to determine compounds responsible for antimicrobial activities.

STATISTICAL ANALYSIS

The statistical analyses was performed according to the fully randomized design and with three replicates for each treatment. The results were analyzed and compared at the 0.05 level of probability using the L.S.D. using the 16

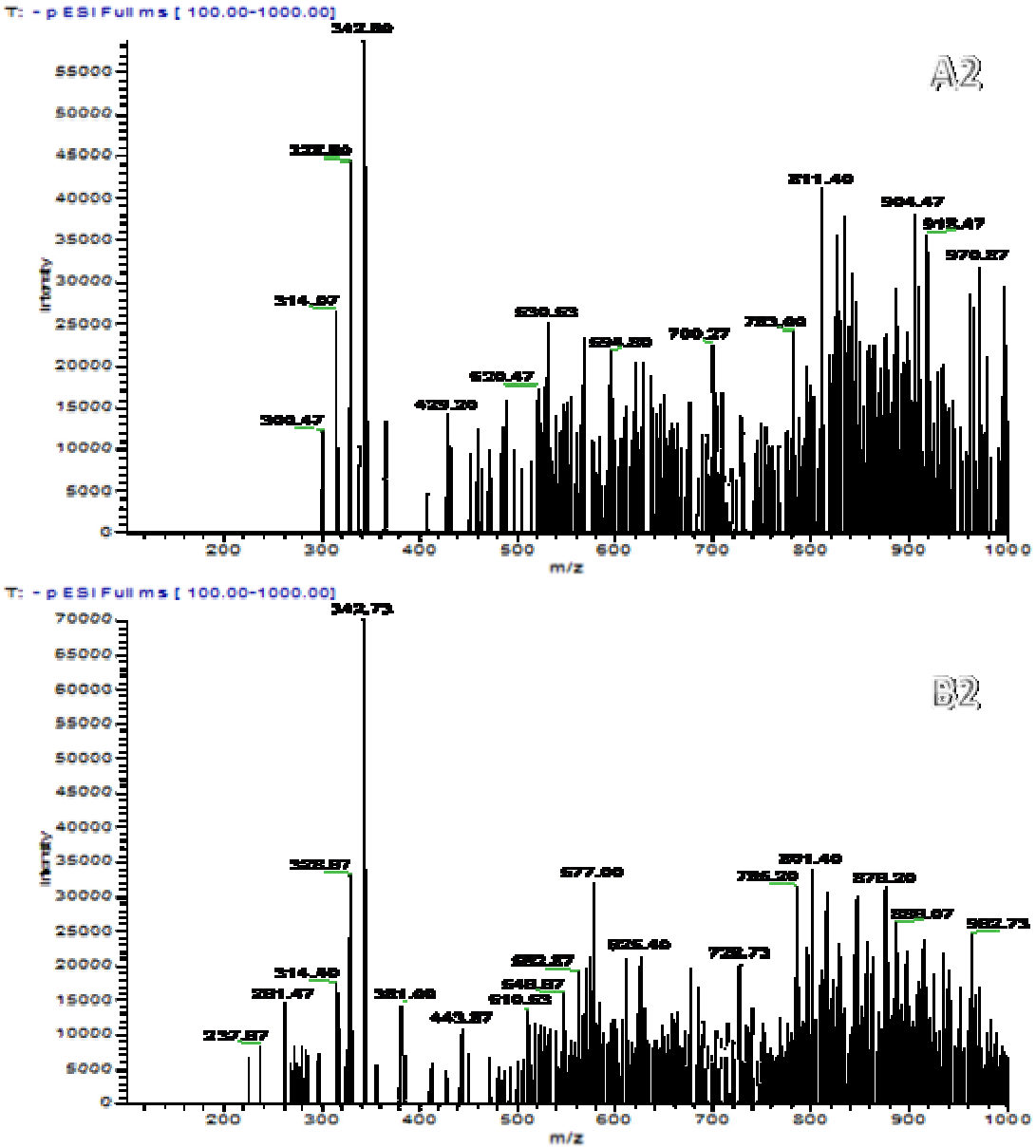


Fig. 2. Chromatograms of A2= Myrrh (*C. myrrha)* and B2=Myrrh (*C. molmol)* by mass spectrum after sex month of storage.

version of SPSS program according to the method of Norusis (1999).

RESULTS AND DISCUSSION

Effects of Myrrha aqueous extracts

Effects of Myrrha aqueous extracts were tested on four microbes using the agar well diffusion method, due to its quality, easiness of performing it and clarity of its results. The results were determined after 24-48hours by measuring the inhibition zones area. The results recorded in table 1 show that the increase in the storage period of myrrha reduced its inhibitive effect on most bacteria tested. It was also noticed that *C. myrrha* lost the inhibitive effect on the growth of *Proteus mirabilis* and *Micrococcus luteus* with all the different concentrations of the myrrha aqueous extracts stored for one month, sex months and one year.

The aqueous extracts of *C. molmol* lost its inhibitive activity against *Proteus mirabilis* only after storage for one year. Through the general averages, it is noted that the inhibition area was 1.55, 1.1 and 0.41cm^2 after storage for one month, six months and one year for *C. myrrha* respectively, while the inhibition are a reached 1.53, 1.11 and 0.76cm^2 for *C. molmol* during the storage periods respectively.

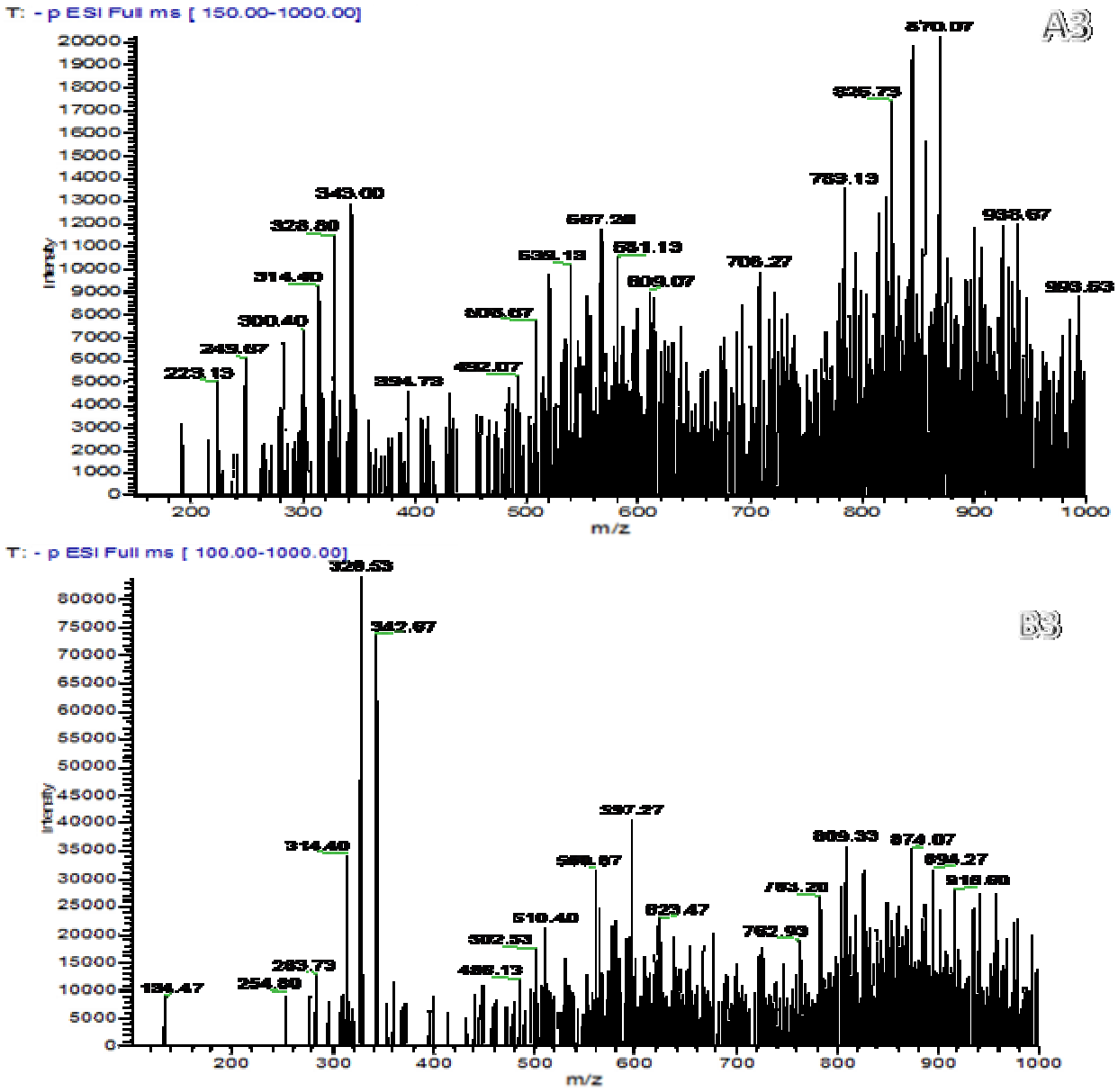


Fig. 3. Chromatograms of A3= Myrrh (*C. myrrha)* and B3=Myrrh(*C. molmol)* by mass spectrum after one year storage.

The greatest inhibition area were recorded when one month old myrrha samples were used with 50% concentration. The inhibited area were as follows: 2.47, 2.43, 2.18 and 1.68cm^2 for *Micrococcus luteus, Neisseria sicca, Pseudomonas aeruginosa* and *Proteus mirabilis* respectively with *C. myrrha*. While the inhibited area was 2.45, 1.99, 1.98 and 1.69cm^2 for *Proteus mirabilis*, *Neisseria sicca, Pseudomonas aeruginosa* and *Micrococcus luteus* respectively with *C. molmol*. The results obtained match the results obtained by other researches including Arora and Kaur (1999), Digraki *et al*. (1999), Okemo *et al*. (2001), Madamombe and Afolayan (2003), Al-Rashedi and Al-Habib (2011) and Akintobi *et al*. (2013).

The positive results show that these extracts contain some anti-microorganisms effective compounds such as the volatile oils, terpenes, phenols, flavonoids and Alsaboninat (Ellof, 1998; Ekwenye and Elegalam, 2005). The bacterial resistance to the tested extracts is due to the composition of the bacteria that resist the antibiotics, especially the thickness of the mucous layer surrounding the cell wall resulting from its adaptation with the excessive and wrong use of the antibiotics. This results in increase in the number of strains that resist antibiotics. These results matched with what is referred to by Ghareeb (2011) on the sensitivity of isolates to the *Staphylococcus aureus* that are distinguished with having mucous layer produced by the resisting isolates that were more

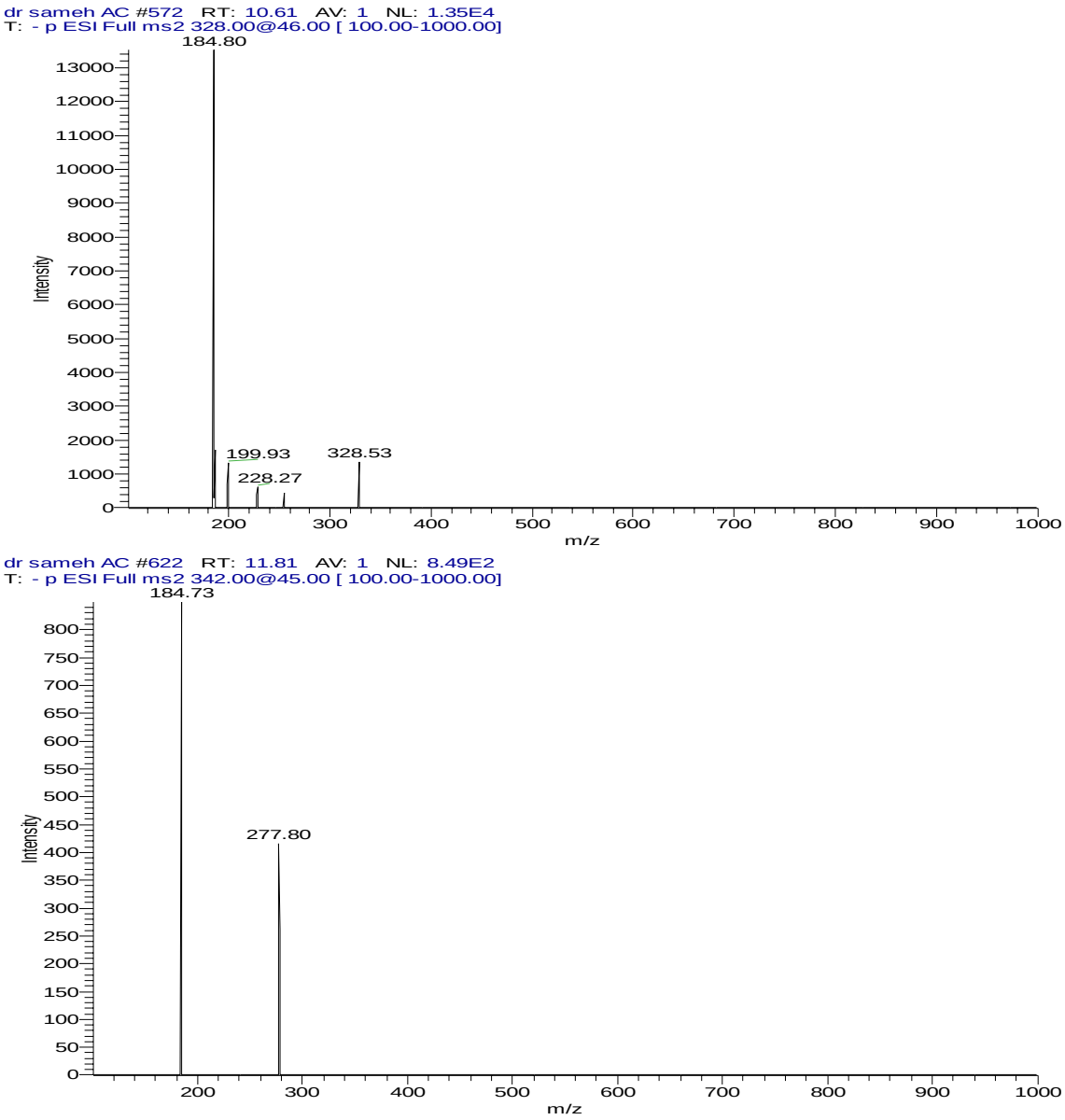


Fig. 4. Chromatograms Individualize the main compound of *C. myrrha and C. molmol* by mass spectrum.

thickness than the layer in the sensitive strains, the mucous layer covers the bacterial cell by some layers to form thin membranes called "Bio-film". Such membranes work as insulators and hinder the influence of the antibiotic thus increasing the resistance property (Kirisits *et al.,* 2007; Stapper *et al.,* 2004). Therefore, the mucous layer is an important factor which enables bacteria to produce resistance to antibiotics causing delay and difficulty in treatment of bacterial infections (Evans *et al.,* 1991). Bayer *et al*. (1992) founded that the mucous layer of *P. aeruginosa* plays an important role in the pathogenicity and the acquisition of resistance. It is one of the factors leading to the appearance of resistance to the antibiotics, and the mucous layer is distinguished with its viscosity and its soft gel composition of little inherence. This layer can surround any type of the bacteria giving it the ability to adhere to other materials. And they find that the bacteria that own capsule, purse or even mucous layer resists the macrophage cells in the human body that is considered as one of the defense lines in the human body.

Chemical composition of myrrha resin in the aqueous extract

The results obtained showed that the aqueous extract of *C. myrrha and C. molmol* contains compounds that inhibit the growth of the tested bacteria In order to know the

Table 2. Inhibition zone (mm) of antibiotic on four microorganisms.

Antibiotic	Diameter of inhibition zone (mm)			
	Micrococcus luteus	*Neisseria siccca*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Gentamicin	1.4	1.55	1.55	1.2
Neomycin	1.9	3.15	2.33	0
Cephalothin	0	2.25	0	0
Cotrimoxazole	0	3.1	1.58	0
Tobramycin	1.1	2.75	1.48	1.5
Carbenicillin	0	0	0	0
Chloramphenicol	0	0	0	0
Polymyxin B	0	1.75	1.26	1.4
Penicillin	0	0	0	1.9
Streptomycin	1.9	1.6	1.7	2.33
Oxytetracycline	1.3	1.75	1.75	1.3
Erythromycin	0	1.65	0,95	0
L.S.D.#	0.006	0.014	0.032	0.006

identity of these compounds, a chemical analysis has been performed for the composition of the myrrha using the method of mass spectrum (Figs. 1-4). It is proved that it contains three compounds known for their antibacterial effects as follows: 2-fluorodiphenylmethane, Tribenzo-1, 2, 3, 4, 5, 6 anthracene and 2-bromo-1-(4-bromophenyl)-Ethanone. The inhibitive effect of the extract may be referred to the existence of the volatile oils that are large terpene single compounds (Cowan, 1999). These oils have the ability to inhibit yeast and this is referred to the ability of the oil to analyze the cell wall. This leads also to the weakening the biological activities in the cell through overlapping with the cytoplasmic membrane function represented by the process of synthesis of protein and this inhibiting and stopping the process. This results also in hindering the process of active transfer of the ions and salts through this membrane (Al-Qaysia, 2008).

Test of examining the sensitivity to the antibiotics

12 types of pharmaceutical bacterial antibiotics were tested in order to know the sensitivity of the tested microbes to see their effectiveness towards these. The results in table 2 show the difference of sensitivity of bacteria tested to the different types of antibodies. The *Micrococcus luteus* was more resistant, and affected only by five of the antibiotics tested followed by *Proteus mirabilis* that affected by six antibiotics only. The growth of *Pseudomonas aeruginosa* inhibited only by eight antibiotics, while not affected by Cephalothin, Carbenicillin, Chloramphenicol and Polymyxin B. *Neisseria sicca* was the most sensitive one of the bacteria tested, resisted three thereof only; namely Carbenicillin, Chloramphenicol and Polymyxin B. the obtained results matched to some extent with the results obtained by Akintobi *et al.* (2013).

Ghareeb (2011) and Vasil (1986) found that the resistance of *P.aeruginosa* and *Staph. aureua* to the antibodies may have resulted by diferrent mechanisms including the production of enzymes able to break down the β-lactamase enzymes or change the permeability of the cell membrane in order to hinder the entrance of the antibiotic to the targeted area as well as its ability to change the metabolic pathways. Brown (1975) refers this to that some world hospital's is restricted to use one antibiotic to treat its patients resulting in appearance of mutant strains resistant to these antibiotics.

In general, the mechanisms followed by microorganisms for survival against microbial antibiotics is still ambiguous and debatable (Okemo *et al*., 2001). On the other hand, the chemical components of the plants play a role in protecting the plants from the microbial attack inside the plant. Some of these components may also be used by humans for protection against microorganisms (Kubo *et al*., 1995). This study recommends that more experiments be performed to test the natural plant components for antimicrobial performance.

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