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Effect of plants extracts on the growth of *Candida albicans* and *Staphylococcus aureus*

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The purpose of this study was to estimate the effectiveness of cardamom, cinnamon, ginger, cloves and myrrha extracts on the inhibition of *Candida albicans* and two isolates of *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus (*MSSA) and in different concentrations*.* The results showed that the aqueous extract of spices have no inhibitory effect on the growth of the tested microbes, while, that two types of the Myrrha (*Commiphora myrrha* and *C. molmol*) aqueous extracts inhibited all the tested microbes. Also, the alcoholic extract of four spices had inhibitory effect on the growth of three pathogenic tested isolates. By performing the chemical analysis for the Myrrha, it was noted that it contains three components known for their antimicrobial effect. These components are: 2-fluorodiphenylmethane, Tribenzo-1,2,3,4,5,6anthracene and 2-bromo-1-(4-bromophenyl)-ethanone. In addition, the activity of 8 types of the bacterial antibiotics used pharmaceutically in order to know the sensitivity of the microbes' tested showed that *S. aureus* (MRSA) and *C. albicans* were more resistant, as it not affected by all the tested antibiotics. As a result, it is more effective to use Myrrha instead of industrial antibiotics.

Key words: *Staphylococcus aureus*, *Candida albicans*, *Commiphora,* 2-fluorodiphenylmethane, 2-bromo-1-(4-bromophenyl)-ethanone, cardamom, cinnamon, ginger, cloves.

INTRODUCTION

Many medicinal plants produce antioxidant, antimicrobial and anti-inflammation properties which protect the host from cellular oxidation reactions and other pathogens highlighting the importance of looking for natural antimicrobial drugs (Farzaei et al., 2014). In addition, adding the spices to foodstuffs is not limited to improving the flavor and test, but this also implies preservative and antibacterial effects on the plant and the human pathogens. While the antimicrobial drugs have been discovered and the effectiveness thereof has been proved remarkably in controlling the bacterial infections, but the absolute effectiveness thereof without restriction or condition cannot be admitted, due to the ability of some pathogens to adapt quickly and to resist many of the discovered effective medicines (Cowan, 1999). Many studies have been performed on the natural ingredients

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in the same, their antimicrobial and antifungal effect has been tested and their effect has been compared with the standard antibiotics (Sakagami et al., 2001; Velickovic et al., 2003). Many researchers in the field of natural antibiotics for microbiology have indicated the importance of using the plant extracts distinguished with the ease of obtaining the same and its low side-effects in comparison with the synthetic antibiotics (Adel and Mahasneh, 1999; De-Boer et al., 2005; Shabana and El-Adly, 2016).

Egyimann et al. (2003) have stated that during the last ten years, the infections resulted from *C. albicans* and systemic infections resulting from the same especially among the immunodeficiency patients. The studies have shown that the resistance of yeast is increasing continuously for the artificial antibiotics that are represented in Azoles Group such as the fluconazole (Rex et al., 1995).

The cardamom, scientific name *Electaria cardamom*, is affiliated with Zingiberaceae Family. Cardamom is used in many and various ways in different foods whether solely or mixed with other spices and it is distinguished with many health benefits; it repels the gases, comfortable for the digestive system, catalysts for the digestion, has beneficial effect on the liver and in resisting the cold, fever and mouth inflammations (Bekel, 2007).

In addition, the cinnamon, scientific name *Cinnamon zeylanicum* is affiliated with Lauraceae, its oil is used as gases repellant, eliminates the spasm, general stimulant, antiseptic, anti-diarrhea, treats diabetes, reduces cholesterol, fat and gum diseases, stimulates blood circulation and it has activity against inflammations (Giday, 2001). Also, it has a potential medical use with regards to its antimicrobial properties, especially antibacterial activity (Nabavi et al., 2015).

Ginger, scientific name *Zingiber officinale*, affiliated with Zingiberaceae, common name ginger is known with its uses in the medical field from antiquity (Mascolo et al., 1998; Akoachere et al., 2002). It has been listed in the medical plants, due to its wide extent of medical uses (Ficker et al., 2003; Habsah et al., 2000). Chemical analysis indicate that the ginger contain effective compounds against certain micro biologics; these compounds are shogaols, zingerone and gingerols (Ernst and Pittler, 2000); while Rehman et al., 2011 stated that it contains essential oils consisting of the compounds sesqiterpenoids, B-sesquiphellandrene, phenylpropanoid, cineol, farnesene, bisabolene.

The clove, scientific name: *Syzygium aromaticum*, in the Myrtaceae Family, common name Carnation is gases repellant, eliminates the spasm and it is one of the stimulants, antioxidants, antipyretic, natural anti-microbial, anesthetic, anti-throat inflammations and dental problems (Lemma et al., 2002).

Myrrh, scientific name *Commiphora myrrha* and *Commiphora molmol*, is in Bruseraceae family, common name: Myrrh; the resin of this plant in used in treating the cuts, bowel disorders, diarrhea, coughing and chest (Ghazanfar, 1994) and it is also used for treating (Serfaty and Itid, 1988). Rahman et al. (2008) stated that resin has been obtained from *C.molmol* which is active against many strains of *Staphylococcus aureus*. Hussien et al. (2011) performed a study in order to test the effect of aqueous plant extracts of cinnamon, cardamom, cloves, thyme, mustard and basil in inhibiting *Candida albicans* and *S. aureus*, they noticed that their ability in the inhibition is different, as they have positively affected the growth of *S. aureus* with rate of 10-20%, while they have no effect on *C. albicans*. Moreover, Takahashi et al. (2011) confirmed that ginger oil is more effective in inhibiting *C. albicans* yeast than the other oils and the minimum inhibiting concentration (MIC) is 200 mg/mL; while, the minimum inhibiting concentration (MIC) for the alcoholic extract of Myrrh which affects strains of *S. aureus* tested is 31.25 and 250 mg/mL (Abdallah et al., 2009). Al Ahmadi (2006) stated that the oils of Commiphora resins are rich in furanosesquiterpenoids compounds in a total number of 20 different compounds from such type. The separated compounds of furanosesquiterpenoids or extracts of Commiphora resin extracts have showed activity that resists the bacteria and fungi and they have anesthetic properties. Therefore, this study aimed at finding chemical materials from natural plant source that have effectiveness in inhibiting the growth of *C. albicans* and *S. aureus* whether they are sensitive or resistant to the known antibiotics and antifungal drugs to avoid human health hazards.

MATERIALS AND METHODS

Plant samples

The plant-samples, *C. myrrha* and *C. molmol*, were collected from retail-shops in Dammam city. The bought samples were kept at 4°C until tested.

Microbial isolates source

There are two isolates of *S. aureus* (MRSA) and *S. aureus* (MSSA) and fungal strain *C. albicans* (yeast, clinical isolate) employed for activity testing, were obtained from Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Dammam. The microbial samples were subculture and kept at 4°C until used.

Preparing the extracts

Aqueous extract

Five grams powder of plant material was weighed in an Erlenmeyer of 200 ml to which 100 ml of distilled water were added which gave the final concentration of 5%. They were soaked at room temperature for 24 h, then filtered using layers of sterilized gauze (Boyraz and Ozcan, 2005).

Essential oils

Four essential oils obtained from perfumery shops in Dammam city,

(commercial producers of plant essential oils (80%) and aromatic substances) were used in this study. From each essential oil (cardamom – cinnamon- Ginger and clove), 2 ml were transferred to 9 ml ethyl alcohol (80%), after that concentrations 55.56, 83.3, 111, 250 and 1000 µ g/ml were prepared according to Prabuseenivasan et al. (2006), but 80% ethanol alcohol without oil, was used as a positive growth control.

Media preparation

Two media were used to evaluate the antimicrobial activity as follows: Nutrient Agar Oxiod was prepared for bacteria and Sabouraud Dextrose Agar Oxoid was used for yeast, which was incubated at 28°C, then transferred 250 ml into flasks and autoclaved at 121°C for 15 min.

Testing the effectiveness of aqueous extracts

The agar well diffusion method was used (Perez et al., 1990). From microbial inoculum, 1 ml was added in a sterile plastic Petri dish and then 10 ml of the medium was poured and left to harden. Then the plates incubated at temperature of 23-30°C for 48 h. The results were recorded by calculating the inhibited area. The inhibition zone was measured in mm (Amade et al., 1994).

Determination of minimum inhibitory concentration (MIC)

The antimicrobial activity of the extracts was determined by 2-fold dilution method using above media and MICs were read in µg/ml after overnight incubation at 37°C (Omura et al., 1993).

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

Chemical composition analysis of myrrha was performed using mass spectrum, the GC–MS analyses of the volatile oils were carried out using a Hewlett-Packard 5890 gas chromatograph coupled to a VG Analytical 70-250S mass spectrometer, to determine the active principle compounds responsible for antimicrobial activities.

Statistical analysis

The statistical analyses were 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. and the 16 version of SPSS program according to the method of Norusis (1999).

RESULTS AND DISCUSSION

Effect of the aqueous extracts of the tested plants

A study on effect of aqueous extracts of the tested spices on the three microbes using agar well diffusion technique was done for its quality, the ease of performing the same and the clarity of results thereof. The results were determined after 24-48 h by measuring the dimeters of inhibition zones. The results recorded in the Tables 1, 2 and 3) showed that there is no inhibiting effect of the growth of the tested microbes for the aqueous extract of cardamom, cinnamon, ginger and cloves; while the aqueous extract of both types of tested Myrrha was showed that there is inhibition zone for the microbes, the subject matter of the study, and that the resistant *S. aureus* (Table 1) was not affected by the first type of Myrrha, while its growth was inhibited by the three first concentrations of the Myrrha of the second type, *C. molmol*. The diameter of inhibition zone reached 0.874, 0.852 and 0.600 mm on the concentrations 1000, 250 and 111 µ g/ml successively. While that the sensitive *S. aureus* (Table 2) was the most affected. The present result showed that the sensitive *S. aureus* was the most affected by two types of aqueous extract of myrrha with a mean inhibition zone of 0.874 to 0.651mm with *C. myrrha* at 1000 and 83.3 MIC (µ g/ml) and 0.874 to 0.826 mm with *C. molmol* at 1000 and 250 MIC (µg/ml), respectively. Also, *C. albicans* has been affected by both types of Myrrha. The inhibited zone reached 0.776 and 0.749 mm for both concentrations of 250 and 1000 MIC (µg/ml) for the first type of Myrrha, while such zone reached 0.883 and 0.725 mm, successively for the second type of Myrrha. This result is in agreement with Rash and Al-Habib (2011) and Akintobi et al. (2013) and it disagrees with Hussien et al. (2011).

Impact of the essential oils of the tested plants

A study on essential oils impact of the tested spices on the microbes studied by the previous method and the obtained results as recorded in the Tables 1, 2 and 3 showed that the effectiveness of the plants essential oils toward the microbes was different according to the combination of the microbe. And *C. albicans* and *S. aureus* sensitively to the antibiotics have shown sensitivity more than the resisting bacteria which has not been affected by the essential oils of all the tested plants. The bacterial sensitivity to the antibiotics has shown a remarkable susceptibility to the essential oils of all the tested plants with all concentrations. The effectiveness of the cinnamon oil was greater followed by the clove then the ginger and finally the cardamom. The yeast was affected generally and its sensation to the cinnamon was greater followed by the cardamom, then the clove and finally the ginger.

The results obtained in relation to the sensation of bacteria and yeast of such extracts are in agreement with that obtained by many researchers (Arora and Kaur, 1999; Digraki et al., 1999; Okemo et al., 2001; Madamombe and Afolayan, 2003 and Akintobiet al., 2013). And disagreement with other such studies were made by Brandi et al. (2006), and Al-Rashedi and Al-Habib (2011) have confirmed that the ethanol extracts of the tested plants have a higher biological effects than the aqueous extracts on the growth of *Candida* sp. however,

Table 1. Effect of some of the aqueous and alcoholic extracts of certain plants on the growth of *S. aureus* (MRSA).

Treatment	Concentration (µ g/ml)	Aqueous extracts		Alcoholic extracts	
		Inhibition Zones*	MIC (µg/ml)**	Inhibition Zones*	MIC (µg/ml)**
Control	0	0.00	-	0.00	-
	1000	0.00		0.00	
	250	0.00		0.00	
Cardamom	111	0.00	-	0.00	
	83.3	0.00		0.00	
	55.56	0.00		0.00	
	1000	0.00		0.00	
	250	0.00		0.00	
Cinnamon	111	0.00	-	0.00	
	83.3	0.00		0.00	
	55.56	0.00		0.00	
	1000	0.00		0.00	
	250	0.00		0.00	
Ginger	111	0.00	-	0.00	
	83.3	0.00		0.00	
	55.56	0.00		0.00	
	1000	0.00			
	250	0.00			
Clove	111	0.00	-		
	83.3	0.00			
	55.56	0.00			
	1000	0.00			
Myrrh (*Commiphora myrrha*)	250	0.00			
	111	0.00	-		
	83.3	0.00			
	55.56	0.00			
	1000	0.874			
Myrrh	250	0.852			
(*Commiphora molmol*)	111	0.500	111		
	83.3	0.00			
	55.56	0.00			
L.S.D.#		0.050	-	0	-

*Mean diameter of inhibition zone (mm); **minimal inhibitory concentration.

it was discovered that anti-microbial compounds derived from plants hamper the growth of bacteria by processes separate from those currently utilized and anti-bacterial and they may have a great therapeutic value for the clinical diseases in resisting bacteria`s strains (Harborne, 1998).

The results of *Candida* and sensitive bacteria are positive, because these extracts contain some effective compounds antibacterial and antifungal compounds that act against these microbes like the volatile oils, turbines, phenols, flavonoids and saponins (Ellof, 1998; Ekwenye and Elegalam, 2005). The resistance of the *S. aureus* (MRSA) against the tested extracts may be referred to what is known as the combination of the same. Especially, the thickness of the mucous layer that surrounds the cell wall that has resulted from its adaptation with the excess and wrong use of the antibiotics resulting in increase in numbers of strains that resist the antibiotics whether those chemical or natural, regarding the sensitivity of 60 isolates of *S. aureus* are shown by having a mucus layer produced by the resisting isolates that was thicker than the layer of the sensitive strains, and the mucus layer covers the bacterial cell acting as insulator prevents the penetration of the

Table 2. Effect of some of the aqueous and alcoholic extracts of certain plants on the growth of *S. aureus* (MSSA).

Treatment	Concentration (µ g/ml)	Aqueous extracts		Alcoholic extracts	
		Inhibition zones*	MIC (µ g/ml)**	Inhibition zones*	MIC (µ g/ml)**
Control	0	0.00	-	0.00	-
	1000	0.00		1.374	
	250	0.00		0.716	
Cardamom	111	0.00	-	0.631	83.30
	83.3	0.00		0.599	
	55.56	0.00		0.00	
	1000	0.00		2.25	
	250	0.00		2.199	
Cinnamon	111	0.00	-	1.900	55.56
	83.3	0.00		1.533	
	55.56	0.00		0.631	
	1000	0.00		1.748	
	250	0.00		0.874	
Ginger	111	0.00	-	0.725	55.56
	83.3	0.00		0.623	
	55.56	0.00		0.573	
	1000	0.00		1.799	
	250	0.00		1.599	
Clove	111	0.00	-	1.574	55.56
	83.3	0.00		1.150	
	55.56	0.00		0.523	
	1000	0.826			
Myrrh	250	0.874			
(*Commiphora*	111	0.675	83.3		
myrrha)	83.3	0.651			
	55.56	0.000			
	1000	0.826			
Myrrh	250	0.874			
(*Commiphora*	111	0.000	250		
molmol)	83.3	0.000			
	55.56	0.000			
	L.S.D.#	0.001	-	0.900	-

*Mean diameter of inhibition zone (mm); **minimal inhibitory concentration.

antibiotic to inside the bacterial cell; it is resistant (Kirisits et al., 2007; Stapper et al., 2004).

Chemical effectiveness of the Myrrha resin in the aqueous extract

The obtained results showed that the aqueous extract of both types of Myrrha contains materials inhibiting the growth of the three microbes. In order to know the identity of such compounds, a chemical analysis was performed for Myrrha combination using the analysis method of mass spectrometry (Figures 1 and 2), as it has been proven that it contains three compounds known for their inhibiting effect as follows: 2-fluorodiphenylmethane, tribenzo-1,2,3,4,5,6-anthracene, 2-bromo-1-(4-bromophenyl)-ethanone. The inhibiting effect of the extract is attributed to the presence of the volatile oils

Table 3. Effect of some of the aqueous and alcoholic extracts of certain plants on the growth of *Candida albicans*.

Treatment	Concentration (µ g/ml)	Aqueous extracts		Alcoholic extracts	
		Inhibition zones*	MIC (µg/ml)**	Inhibition zones*	MIC (µg/ml)**
Control	0	0.000	-	0.000	-
Cardamom	1000	0.000	-	2.235	55.56
	250	0.000		2.199	
	111	0.000		2.077	
	83.3	0.000		1.250	
	55.56	0.000		0.000	
Cinnamon	1000	0.000	-	2.390	83.30
	250	0.000		1.250	
	111	0.000		0.820	
	83.3	0.000		0.709	
	55.56	0.000		0.000	
Ginger	1000	0.000	-	1.599	83.30
	250	0.000		1.324	
	111	0.000		1.184	
	83.3	0.000		0.736	
	55.56	0.000		1.599	
Clove	1000	0.000	-	1.749	55.56
	250	0.000		1.450	
	111	0.000		1.249	
	83.3	0.000		1.033	
	55.56	0.000		0.000	
Myrrh (*Commiphora myrrha*)	1000	0.776	250		
	250	0.749			
	111	0.000			
	83.3	0.000			
	55.56	0.000			
Myrrh (*Commiphora molmol*)	1000	0.883	250		
	250	0.725			
	111	0.000			
	83.3	0.000			
	55.56	0.000			
L.S.D.#		0.003	-	0.222	-

*Mean diameter of inhibition zone (mm); **minimal inhibitory concentration.

which are large single turbine compounds (Cowan, 1999).

These oils have the ability to inhibit the growth of yeast types and this is referred to the ability of the oil to analyze the cell wall and this also leads to weakening of the vital activities inside the cell by interfering with the function of the cytoplasmic membrane represented in the process of building the protein and thus inhibiting and stopping the process and also hindering the process of the effective transferring of the ions and salts through such membrane (Al-Qaysi, 2008).

Test for examining the sensitivity to the antibiotics

Eight pharmaceutically used bacterial antibiotics have

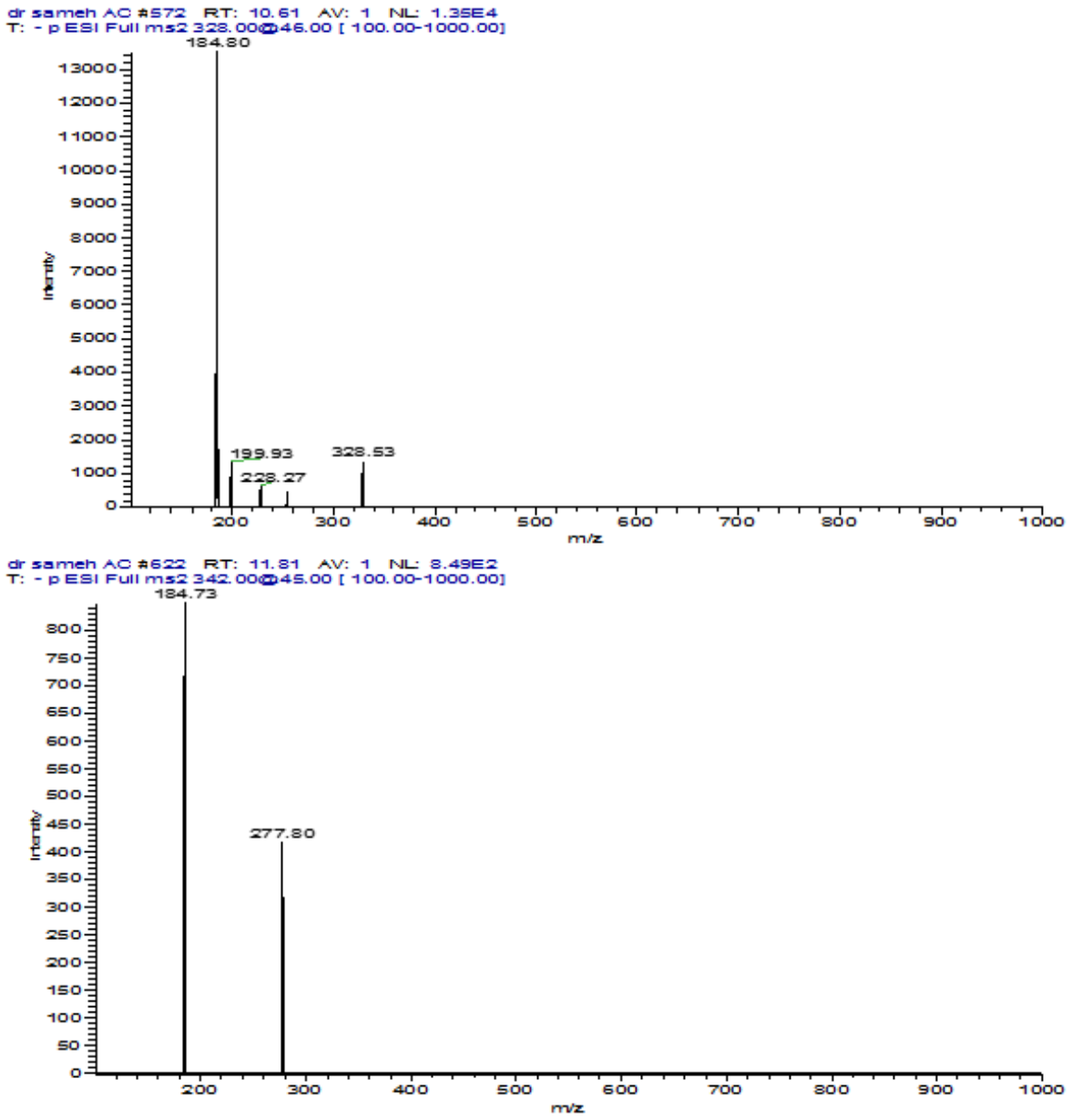

Figure 1. Chromatograms showing the main compound of *C. myrrha and C.molmol* by mass spectrum.

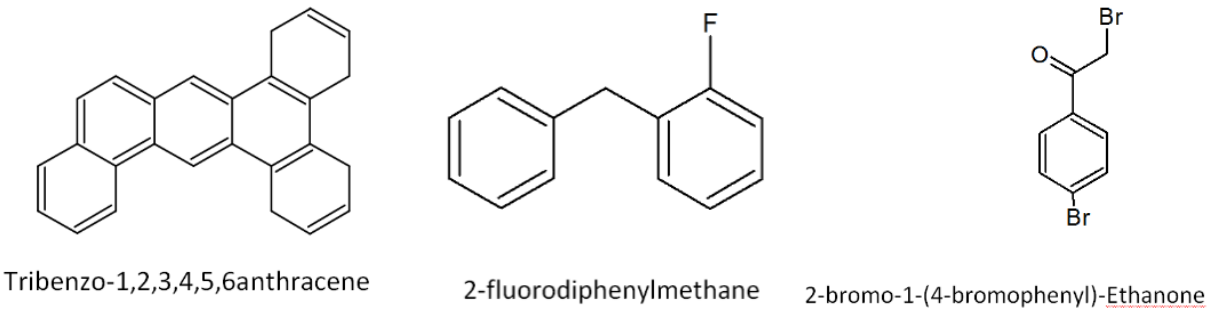

Figure 2: The chemical structures of three bioactive compounds derived from aqueous extract of Myrrh

Table 4. Impact of effectiveness of some antibiotics on the tested microbes.

Antibiotics	Mean diameter of inhibition zone (mm)		
	Candida albicans	***Staphylococcus aureus* (MRSA)**	***Staphylococcus aureus* (MSSA)**
Oxytetracycline	0.000	0.000	0.533
Gentamicin	0.000	0.000	1.350
Carbenicillin	0.000	0.000	0.000
Cotrimoxazole	0.000	0.000	0.000
Cephalothin	0.000	0.000	0.000
Tobramycin	0.000	0.000	0.175
Chloramphenicol	0.000	0.000	0.340
Polymyxin B	0.000	0.000	0.433
L.S.D.#	0.000	0.000	0.011

been used in order to know the sensitivity of the same microbes towards these antibiotics. The results shown in Table 4 show the resistance of *S. aureus* (MRSA) to all tested antibiotics. The obtained results are in agreement with what been obtained by Akintobi et al. (2013). Moreover, all the tested antibiotics have no effect on *C.albicans*. This is not odd, because the combination of the bacterial cells is different from the yeast, while five of the antibiotics affected the growth of the sensitive bacteria *S. aureus*, as the diameter of the inhibited zones reached 1.350, 0.550, 0.533, 0.433 and 0.400 mm for each of Gentamicin, Tobramycin, Oxytetracycline, Polymaxin B and finally Chloramphenicol, while the three antibiotics carbincilin, co-trimoxazole and sifalothin had no effect on the growth of the bacteria. Vasil (1986) stated that the resistance of *P. aeruginosa* to the antibiotics may result from different mechanisms including the production of enzymes able to destroy the antibiotics like the Betalactymize or to change in the penetration of cell membrane in order to prevent the access of the antibiotic to the target region as well as its ability to change the metabolic routes. Brown (1975) thinks that the reason for this is that some of the hospitals are restricted to the use of one antibiotic for curing their patients and this has resulted in the appearance of mutant strains resistant to these antibiotics.

In general, the mechanisms used by the micro-organisms to survive against the activity of the microbial antibiotics are still obscure and subject to discussion (Okemo et al., 2001). On the other hand, the chemical components of such plants may play a role in protecting the plants from the microbial attack inside the plant; however, some of them may have value as natural chemical materials used for defending the human body against the attack of the germs (Kubo et al., 1995). Therefore, this work recommends the performance of more studies, through which the natural effective components can be extracted and insulated from the plant which may act in saving the life of many peoples. In addition, this will lead to assistance in finding the alternatives in facing the appearance and spread of the strains that resist the known microbial antibiotics.

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Conflict of interests

The author has not declared any conflict of interests.

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