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Study the Effect of Some Natural Oils to Increase Some Antibiotics Activates towards *Aeromonas Hydrophila*

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Abstract: *Aeromonas hydrophila* was taken from the environment laboratory of Science College of Baghdad University. the isolate was confirmed by conducting some agricultural and biochemical tests, the results of the diagnosis shown the isolate refair to *A. hydrophila*. Sesame oil and olive oil used as treatment and compared oil activity with different types of antibiotic. The results appeared high activity for Sesame oil and olive oil that gavethe excellent result as treatment in comparison with antibiotic , the results showed a significant difference in the isolation response to the types of antibiotics and in the case of using natural oils under study as medium support , the results showed there is a significant increase in the effectiveness of the Amikacin (AK) and Doxycycline(DOX) towards the isolate that growth in medium supported by different concentration of olive oil or sesame oil , while the results are not significant in the experiment to use it as antagonists cofactor to increase their effectiveness toward the isolate that grew in normally condition

Keywords: *Aeromonas hydrophila* of water, Olive oil, Sesame seed oil, Antibiotic

Introduction

The genus *Aeromonas* is a member of the family *Aeromonadaceae* that are primarily aquatic organisms found in water. Aeromonas infections are one of the most common bacterial diseases diagnosed in marine and cultured freshwater fish. It is found in diverse habitats, including soil, widely in fresh and saltwater also frequently found in chlorinated and nonchlorinated drinking water, and is pathogenic to warm and cold-blooded animals. Direct contact with contaminated water and soil is the most frequent cause of gastrointestinal and wound infections in humans (Hawraa and Hazim, 2014). *A. hydrophila* (*Aeromonas hydrophila*) has been reported to be associated with the epizootic ulcerative syndrome and other infections in fishes in South East Asian countries, Malayasia, Sri Lanka, Japan and India (Surya *et al*., 2014). Some studies have inculpated these organisms in gastroenteritis ranging from mild diarrhea to life-threatening cholera-like illness in children and adults, wound infections, meningitis, broncho-pulmonary infections and osteomyelitis (Figueras*et al*., 2007) , liver and kidney necrosis and ulcer in different sites of the body (paniago*et al*.,1990)

Major clinical manifestations of invasive *Aeromonas* infections are primary bacteremia, hepatobiliary tract infections, and soft tissue-infections. Most human diseases were reported to be associated with three species, i.e., *A. hydrophila*, *A. veronii*, and *A. caviae*. With the changing taxonomy, the importance of some species in clinical infections increases. For example, *A. dhakensis*, previously named A. aquariorum or *A. hydrophila* sub. dhakensis, was often

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recognized as *A. hydrophila* by

the current phenotype-based identification system (Aravena *et al.*, 2011 ; Chen *et al.*,2014 ; Figueras*et al*., 2009). Rapid and reliable identification of pathogenic microorganisms, including the above-mentioned Aeromonas, is important for surveillance, prevention, and control of food-borne diseases. The established methods for bacterial identification in clinical microbiology are often time-consuming and laborious. Time is required for purification and the identification of pathogenic bacteria (Dieckmann*et al*., 2008). *A. hydrophila* have many mechanisms to resistance antibiotics particularly to lactin, ampicillin, penicillin and nanocin and it also has many virulence factors such as B-hemolysin and cytotonicenteroxin.(Jongjareanjai, *et al*. 2009). The presence of species-related disease syndromes, coupled with differences in antimicrobial susceptibilities among the species, strongly suggests that conventional identification and antimicrobial susceptibilities should be determined for clinical aeromonad isolates (Işın and Simge , 2013).

Sesame oil has several benefits so its uses as traditional therapy to inactivate inflammation because having antimicrobial activity and contains non-saturated fatty acids like linoleic acid and oleic acid and flavonoid compounds which work as antioxidant and has synergistic effect to increase the activity of antibiotics toward different microbes (Khan *et al*,2015) and has an inhibitory effect against capsular formation, motility, urease production, gelatinase and biofilm (Mohammed . Fatimah Abdulazeez,2017), Tafesh *et al*, 2011 explained that olive oil contains many essential fatty acids including much-unsaturated fats, in addition to flavonoids and phenols the most important of which is hydroxytyrosol and a small percentage of tritevpene works together antibacterial agent and slows the growth of resistant *Staphylococcus eureus* (methilin – resistant staphylococcus aureus .(MERS)

Materials and methods:

One sample of nonsterile water was obtained from the environment laboratory of science college of Baghdad university. The bacteria (*A. hydrophila*)was confirmed by taken a water sample and cultured onto thiosulfate citrate bile salts sucrose agar (TCBS) and MacConkey agar (MC), the plates were incubated overnight. Morphological colony characteristics were recorded on these media for identification of *A.hydrophila* and microscopic properties by Gram's stain was used to examine the isolated bacteria such as gram reaction, shape, and motility (Jawetz*et al*., 2007). While Biochemical tests used Oxidase test, Catalase test (Hydrogen Peroxide 3%), Indole Production test, Motility test, urease test, were all these tests or results according to (Hawraa and Hazim, 2014). Also, the API20E system was carried out according to the procedure of (Biomerix company, France).

Antibiotics susceptibility test**:**

The antibiotic susceptibility test was done on*A. hydrophila* by using standard antibiotic discs diffusion techniques (Cruikshank *et al*., 1975). A total of 5 types (1= Doxycycline (Dox ?) ,2 = Trimethoprim / Sulfamethoxazole (SXT 25), 3= Ceftriaxone (CRO 30), 4= Amikacin (AK 30) , 5= cefepime (feep30)) of antibiotics disc (Hi-media) were used for antibiotic sensitivities study. The antibiotic discs were then put on the sensitivity medium plates with the help of sterile forceps, then incubated at 37 C for 24 hours. The results for sensitivity based on the size of the zone of

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bacteria growth inhibition CLSI, (2010).

The impact of natural plant oils

First: Synergistic effect between natural oils and antibiotics.

This test was conducted to show the effect interaction of natural oils (olive .seseam Local oils were supplemented by the special plant to produce in Al-Anbar/Iraq, where it was obtained purely.) treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils) with antibiotic . to increase the sensitivity isolate a.h , the bacteria cultured by spread on Muller Hinton agar and the antibiotics discs which saturated with oils (treatment) then put on the surface of media and incubated in 37C for 24h. The inhibition zone was estimated and compared with the control. (Cruikshank *et al*., 1975)

Second : Booster effect with media

- **The effect of the natural oils as an enhancer in the response to antibiotics**
- Sesame oil and olive oil were added 100 µl |ml with to nutrient broth dimethylsulfoxide (DMSO) 70%. Control was treated in the same method but without adding oils. Culture media was inoculated with an equal volume of isolate and then incubated at 37 C for 24 hours according to (Mohammed and Fatimah Abdulazeez,2017). A sensitivity test was done according to (Cruikshank *et al*., 1975) for isolate under study in different concentrations of oils and antibiotics discs by diffusion on Muller Hinton agar.

Determination minimum inhibitor concentration (MIC):

The Resazurin based microtiter dilution assay (RMDA) with some modulations (Chhillar and Gahlaut, 2013), under sterile conditions, place 100 microliters of nutrient broth that mixed with (0.5% Tween 80) in each well of the wells in the slide, and then put in the first row 100 microliter of the dissolved oil at a concentration (10/2 volume/volume) DMSO, and then transfer 100 microliters from each well to the hole followed by to get a double oil chain dilution graduate from 1/2,1/4 to 1/128.

10 microliter was added for Each well from of 1.5 X 108 CFU/ml, in each plate there were control columns: column (2)

Result and discussion

Isolation and identification

The isolates appeared on MacConkey agar as yellowish opaque colonies, smooth, raised, convex, and round in shape. They were gram-negative, rod-shaped bacteria.The biochemical results were showed that bacteria were oxidase-positive, motile, and was capable of producing acid and gas from different sugar such as maltose, sucrose, and dextrose. The isolates showed positive growth at 37 °C but no growth found at 4°C and 40.The commercially API-20E microbiological kit was used to confirm the diagnosis of bacteria and according to its results, it has been shown that these

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bacteria refer to *A. hydrophila*.

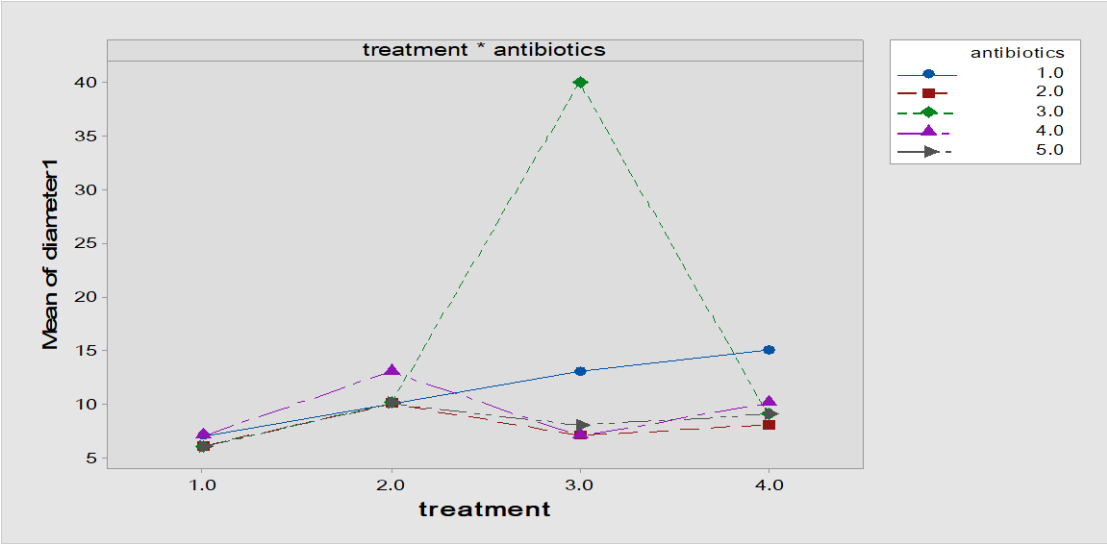
The impact of natural plant oils

First : Sensitivity test and Synergistic effect between Natural oils and antibiotics towards bacterial isolate.

The results of the sensitivity test showed that the isolate (*A. hydrophila*) under test was resistant to the antibiotics DOX, SXT, CRO, AK, Feepwith out any significant result.

The analysis of all variance tables for this experiment indicates that treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils)and antibiotics have no significant effect on the response variable (diameter). That is, the different diameters obtained from this experiment can be attributed to the chance factor (figure 1)

Fig.1 The diameter of the inhibition zone of *A. hydrophila* the results show interaction between treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils) and antibiotics(1= Doxycycline (Dox30) ,2 = Trimethoprim / Sulfamethoxazole (SXT 25), 3= Ceftriaxone (CRO 30), 4= Amikacin (AK 30) , 5= cefepime (feep30) as in agar diffusion method



Second : Booster effect with media

1- The effect of the sesame seed oil as an enhancer in the response to antibiotics

The results appeared the means of diameter were significantly different according to the types of antibiotics used in the experiment.

The graph of main effects for the means diameter according to the types of antibiotics revealed that means diameter for antibiotics types (dox30,AK30) were significantly higher than of the other means.(**Figer 2)**

Fig. 2 antibiotic sensetivity test (1= Doxycycline (Dox ?) ,2 = Trimethoprim / Sulfamethoxazole (SXT 25), 3= Ceftriaxone (CRO 30), 4= Amikacin (AK 30) ,

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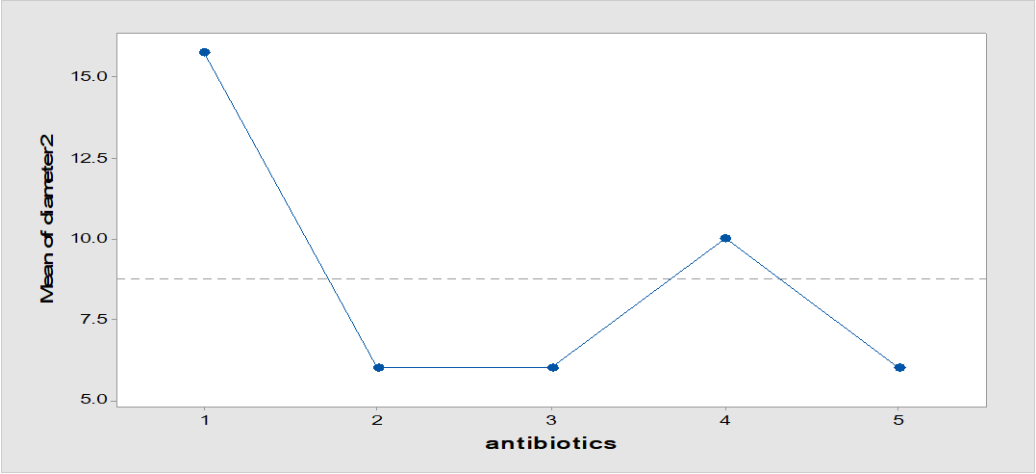
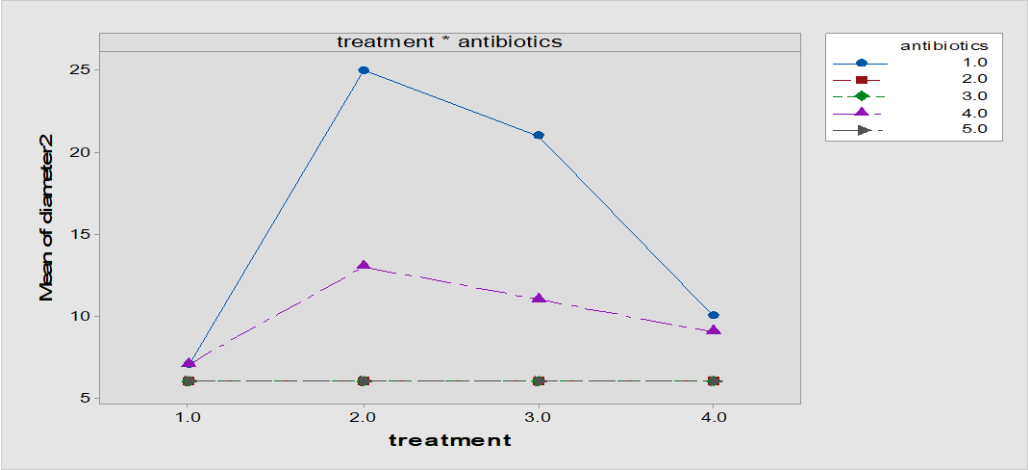
5= cefepime (feep30)) for *A. hydrophila* grown in impact media by sesame seed oil



The interaction plot revealed means diameter for antibiotics types (DOX30, AK30) accounted for essential increment as mentioned previously when compared with respect to the types of treatment whereas means diameter for all other types of antibiotics has

Fig. 3 The diameter of the inhibition zone for *A. hydrophila* grow in impact media by sesame seed oil the resulte show interaction between treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils)and antibiotics(1= Doxycycline (Dox 30) ,2 = Trimethoprim /Sulfamethoxazole (SXT 25), 3= Ceftriaxone (CRO 30), 4= Amikacin (AK 30) , 5= cefepime (feep30)



The effect of the olive oil as an enhancer in the response to antibiotics

The results showed there are significantly different according to the types of antibiotics whereas there is very weak evidence that means diameter were significantly different according to the types of treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils) with antibiotics types (DOX30, AK30) where have the highest means diameter in which they significantly different from other antibiotics figure 4.

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Fig. 4 antibiotic sensitivity test of *A. hydrophila* (1= Doxycycline (Dox ?) ,2 = Trimethoprim / Sulfamethoxazole (SXT 25), 3= Ceftriaxone (CRO 30), 4= Amikacin (AK 30) , 5= cefepime (feep30))test for *A. hydrophila* grown in impact media by olive oil

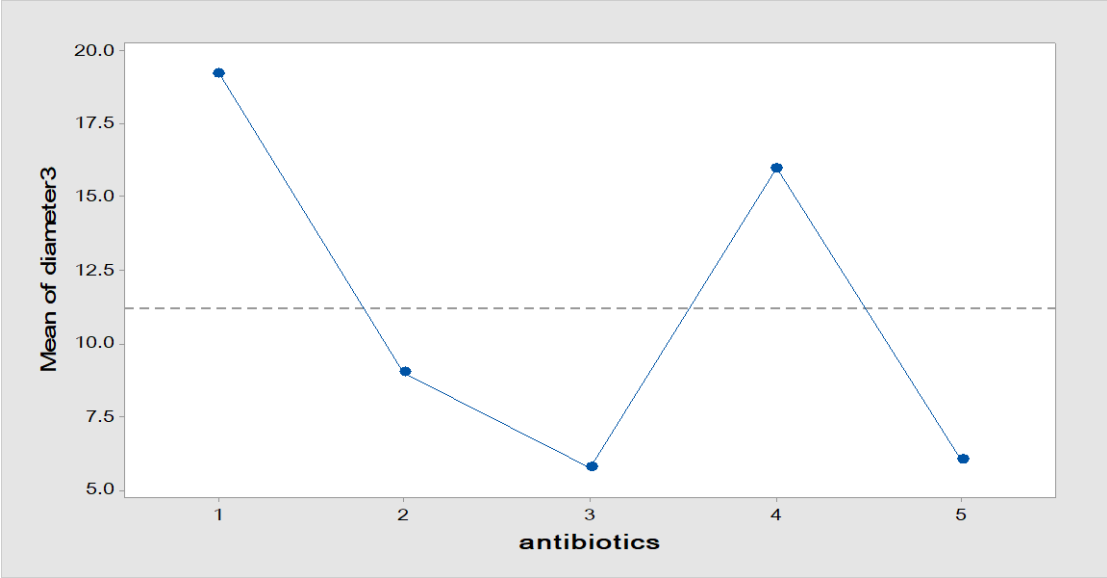
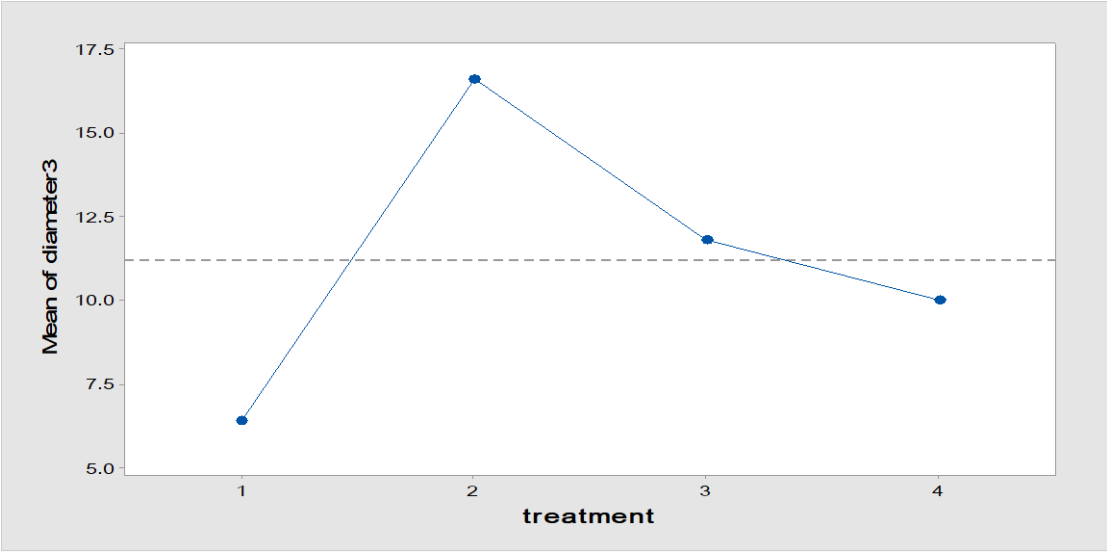


Figure five showed that the means diameter of treatments for DOX, AK appeared the lowest means when compared to other means. Nevertheless, such difference is only accepted at a p-value less than 0.1 which is not very acceptable.

Fig. 5 The diameter of the inhibition zone for *A. hydrophila* grow in impact media 0live oil show effect of treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils) as in agar diffusion method



The graph of the interaction showed that antibiotics SXT, AK were not overlapped with each other and that essentially means that these two antibiotics are significantly different. Moreover, the graph shows that antibiotics DOX and SXT were overlapped constructing one

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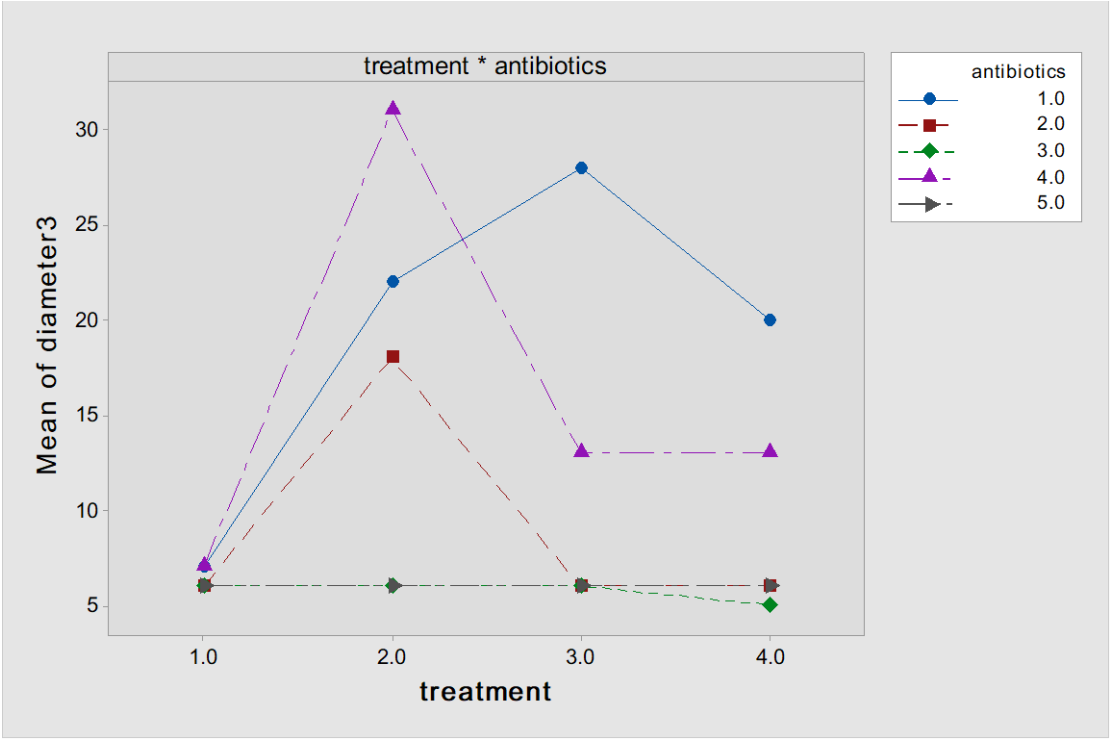
group, and all other antibiotics overlapped with each other constructing another group. Accordingly, antibiotics DOX and SXT are accounted for similar means, and antibiotics CRO, AK, and **Feep** accounted for similar means diameter (figure 6).

Fig.6 The diameter of the inhibition zone of *A. hydrophila* grow in impact media by olive oil show interaction between treatment (1= control 2= sesame seed oil 3= olive oil 4=both oils)and antibiotics(1= Doxycycline (Dox 30) ,2 = Trimethoprim / Sulfamethoxazole (SXT 25), 3= Ceftriaxone (CRO 30), 4= Amikacin (AK 30) , 5= cefepime (feep30) as in agar diffusion method



Tafesh*etal*. (2011) explained that olive oil contains many essential fatty acids, including monounsaturated fats, in addition to flavonoids and phenols, the most important of which is hydroxytryosol, and a small percentage of Tritevpen effected together and slows the growth of resistance.

the results are consistent with Harris 2011 who confirmed that olive oil has an inhibitory effect on some types of bacteria, both negative and positive bacteria for the gram stain, and he explained this to the fact that some natural oils reduce the production of some virulence factors such as biofilm and some enzymes responsible for anti factors biotic resistance

Alheety 2015 explained that the treatment with olive oil reduces the production of some harmful factors such as biofilm, in addition to changing some of the physiological characteristics in the outer layers of the wall or the cytoplasmic membrane, which increases sensitivity change of isolates to antibiotics and various external

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The result showed that sesame oil has activated function towards increased the sensitivity of isolate bacteria this response may be because their intrinsic properties with cell surface permeability as well as to the effect of the active substances on the cell wall and their ability to penetrate and effect in organule (Saranraj and Durga 2017).it have phenolic group binding or deposit water soluble protein can.cause stop functions by inhibiting enzymes or destroyed permeability of plasma membrane, forming complexities with the cell wall . It has the ability to connect with other compounds called nonspecific force such as hydrogen bonds and covalent bonds which explains its non-specialized activities in inhibiting different types of bacteria and fungi (Alfaham2016)

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