

Comparison of Antimicrobial Activity of Olive Leaves Extracts and Apple Cider Vinegar against Bacterial isolates Obtained from Otitis Media

مقارنة الفعالية التضادية بين مستخلص أوراق الزيتون Olive Leaves وخل التفاح Apple Cider Vinegar تجاه بعض البكتريا المعزولة من التهاب الإذن الوسطى Otitis Media
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Abstract:-

The present study included collected 120 sample from patients with otitis media, who attended to consultants of Ear nose throat in AL-Hakeem hospital in Najaf city. Through the period from (8/10/2017-8/1/2018).

The results revealed that the *Pseudomonas aeruginosa* was the most common bacterial isolates (45) 37.5%, followed by *Staphylococcus aureus* & *Acinetobacter baumannii* which constituted (25) 21%, (16) 13% respectively. Test antimicrobial activity of olive leaves extracts and apple cider vinegar against pathogenic bacteria causing otitis media using agar well diffusion method. This result indicated a significant effect ($p < 0.05$), among the microorganism *Proteus* sp which showed most sensitive to cold olive leaves extract range 10-28mm, both *Staphylococcus aureus* and *Pseudomonas fluorescense* showed to high sensitivity boiled olive leaves extract range from 14-28mm, while *Pseudomonas aeruginosa* & *Proteus* sp at high levels of inhibition range 15-30mm. in comparison apple cider vinegar The maximum inhibition zone of Gram negative bacteria was observed against *Escherichia coli*, *Acinetobacter baumannii* 20-37mm, another bacteria include (*Pseudomonas aeruginosa*, *Pseudomonas fluorescense*) & *Proteus* sp the inhibition zone range 15-35mm & 15-30mm respectively, while the observed maximum inhibition zone of Gram positive bacteria 15-30mm, This study shows that Apple cider vinegar consider more effect in the control of otitis media pathogens than olive leaves extract.

Keywords: olive leaves extract, apple cider vinegar, antimicrobial activity, pathogenic bacteria isolate.

الخلاصة :-

شملت هذه الدراسة جمع (120) عينة من المرضى الذين يعانون من التهاب الإذن الوسطى والمراجعين إلى استشارية الأنف والإذن والحنجرة لمستشفى الحكيم في محافظة النجف الأشرف للفترة من (2017/10/8 – 2018/1/8). أظهرت النتائج أن *Pseudomonas aeruginosa* كانت أكثر العزلات البكتيرية شيوعاً إذ تمثلت بنسبة (45) 37.5%، تليها *Staphylococcus aureus* و *Acinetobacter baumannii* التي تمثل (25) 21%، (16) 13% على التوالي. تم اختبار الفعالية التضادية لمستخلص أوراق الزيتون وخل التفاح ضد البكتريا المسببة لالتهاب الإذن الوسطى باستخدام طريقة الانتشار بالحفر. وأوضحت النتائج بأن هناك فروق معنوية عند مستوى احتمالية ($p < 0.05$) من بين العديد من الإحياء المجهرية أظهرت بكتريا *Proteus* sp أكثر حساسية للمستخلص البارد لأوراق الزيتون إذ تراوحت مناطق التثبيط من 10-28ملم. بينما أظهرت *Staphylococcus aureus* و *Pseudomonas fluorescense* أكثر حساسية للمستخلص الحار لأوراق الزيتون إذ تراوحت مناطق التثبيط من 14-28ملم. في حين لوحظ ارتفاع مناطق التثبيط للمستخلص الكحولي لأوراق الزيتون تجاه بكتريا *Pseudomonas aeruginosa* و *Proteus* sp من 15-30ملم. مقارنةً بخل التفاح لوحظ إن الحد الأقصى لمناطق التثبيط للبكتريا السالبة لصبغة كرام والمتضمنة *Escherichia coli*, *Acinetobacter baumannii* ارتفعت من 20-37ملم، بينما البكتريا الأخرى تضمنت (*Pseudomonas aeruginosa*, *Pseudomonas fluorescense*) و *Proteus* sp إذ تراوحت مناطق التثبيط من 15-35ملم و15-30ملم على التوالي. وقد تبين من هذه الدراسة أن خل التفاح له القدرة في السيطرة على البكتريا المسببة لالتهاب الإذن الوسطى أفضل من مستخلص أوراق الزيتون. **الكلمات المفتاحية:** مستخلص أوراق الزيتون، خل التفاح، الفعالية التضادية، عزلات البكتريا المرضية.

Introduction:-

Otitis media (OM) is an inflammation of middle ear which is associated anatomically and pathologically with upper respiratory tract therefore the nasopharynx is a natural reservoir for numerous bacterial species including a virulent bacteria and middle ear pathogens of healthy persons , 20- 40 % accompanied infection together [1].

More than 50 % of the cases of otitis media caused by bacteria, in addition *Mycoplasma pneumonia*, *Chlamydia trachomatis*, fungi, and viruses, may cause otitis media [2]. many of bacterial associated with otitis media different according to the type of infection, *S. pneumoniae*, *M. catarrhalis* and non *H. influenzae* are mainly common bacterial of acute otitis media [3].

P. aeruginosa, *Proteus* spp and *S. aureus* are major pathogen of chronic suppurative otitis media [4]. Revealed researchers have OM to an infection comprise in the middle ear with complex infective demonstration and inflammatory conditions. Based on difference in their presentation and treatment, OM are grouped as acute OM (AOM), OM with effusion (OME), chronic suppurative OM (CSOM) and cholesteatoma with associated complications as mastoiditis, brain abscess formation, meningitis, and sigmoid sinus thrombosis [5].

Attributed to some plants to have the antimicrobial efficiency in treating diseases has been beyond belief. due to their popular use as remedies for several infectious diseases, searches for plants containing antimicrobial substances are recurrent [6]. identified Olive leaf extract (OLE) antimicrobial and antibacterial activity and high antioxidant, OLE is very effective against a variety of diseases, such as hypertension coronary, artery disease,, arrhythmia, high cholesterol level, diabetes, overweight, cancer, osteoporosis, herpes, colds and influenza, and some bacterial, fungus and yeast infections [7]. in *vitro* research demonstrated the efficacy of olive leaf extract against a wide range of pathogens, including *Escherichia coli*, *Streptococcus pyrogenes*, *Pseudomonas fluorescens*, *Helicobacter pylori*, *Compylobactor jujuni*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhimurium*, *Erwinia carotovora*, *Candida albicans* and *Plasmodium falciparum* [8]. Although consider the cider style are very diverse and difficult to categorize, the type of apple juices used and the degrees of sweetness depending them , from additional dry to sweet, and alcohol content, ranging from 1.2–8% (v/v), describe cider can be as a fermented alcoholic beverage made from apple juice [9]. distant from its anti-bacterial property, it also possesses anti-fungal, anti-viral, anti-cancer properties. It has diverse benefits like anti-aging, eliminating bad cholesterol; Polyphenols such as chlorogenic acid which is found in high levels in apple cider vinegar improve health by preventing cardiovascular diseases and could inhibit oxidation of LDLs [10], and helps in weight reduction. The aim of this study to Isolate pathogenic bacteria from patient with suffering from Otitis media and Determine the antibacterial activity of Olive leaves extract and apple cider vinegar against pathogenic bacteria.

Materials and methods:-

Specimen collection & Culture:

During the period from (8/10/2017-8/1/2018), collection 120 specimens from patients who suffering from Otitis Media. All samples were collected from out patients who attended to consultants of Ear, nose, and throat in AL-Hakeem hospital in Najaf city.

Collected samples were inoculated on Nutrient agar, Blood agar, Mannitol salt agar, MacConkey agar and incubated aerobically at 37°C for 24 hours. Colonies on the culture media were selected and identified according to standard bacteriological and biochemical criteria [11].

Preparation of Olive Leaves Extracts:-

1. Olive Leaves Collection

Olive leaves were collected from home garden and washed to remove impurity such as soil and then dry in air for 5-7 days , with daily continuous monitoring to prevent rot and then taken the olive leaves and crushed by electric grinded, then stored in sterile dark bottle until use.

2. Aqueous Extracts: This method was used according to [12].

❖ Cold Aqueous extract

One liter of distilled water was added to 50gm of Olive leaves powder and place on the shaker to be solved thoroughly. The extract was filtered throughout Whattman filter paper (No,1) to remove any un dissolved particles. Finally, obtained extract was sterilized during bacterial filtration (0.22 Mm), and dried by using drying oven under vacuum at a temperature of about 20°C to 85°C.

❖ boiled Aqueous extracts

Twenty grams of powder were flooded in 200 ml of boiling water with 100°C for 30 minutes. The extract was filtered throughout sterile filter paper and the solid parts are strained out. The filtrate was concentrated by using drying oven at a temperature of about 20°C to 85°C. These extract was filter through bacterial filter (0.22 Mm) before use.

3. Ethanolic Alcohol Extracts

Twenty grams of Olive leaves powder are cover flooded 200 ml aqueous alcohol solution (70%) in contact which remains the leaves for at least 4 hours and is then exhausted. This procedure is repeated at least two more times, and the exhausted extract are combined, by the use distillation under vacuum to concentrated, and dry by using oven under vacuum, to obtain a powder containing about 30-40% by weight olive leaves. The steps of the extraction are conducted at a temperature of about 20°C to 85°C according to [13].

4. Concentration of plants extracts

Stock solution was prepared for olive leaves extract by dissolving 1000 mg of dried extract with 1 ml of distilled water for aqueous extract and ethanol for alcohol extract, so the final concentration of extract would be 1000 mg/ml, from this stock solution other concentration were prepared (200,400,600,800,1000)mg/ml. While apple cider vinegar was prepared by intake 10ml from cider with 100ml distilled water so the final concentration would be 100% ml/ml, from this stock solution other concentration were prepared (20,40,60,80,100)% ml/ml. which was used against test bacteria [14].

Assessment of antimicrobial activity:

The assessment of antimicrobial activities of Olive leaves extract and apple cider vinegar by use agar well diffusion method as described by [15]. Mueller-Hinton agar plates were surface-inoculated with suspensions of the tested bacteria adjusted to with 0.5 McFarland standard and the inoculate were spread over the surfaces of plates by use sterile cotton swabs to remain for 5 -15 minutes at room temperature to dry. Cut media into five wells (6mm diameter) by cork borer and put 100µl of Olive leaves extract and apple cider vinegar (The plates were performed in triplicates), incubate at 37°C for 24h , each extract was noted for inhibition zone for all isolates. The diameters of the zone of inhibition were measured in millimeter (mm).

Statistical Analysis:-

Data of the study were analyzed using T- test by using Statistical Package for the Social Sciences (SPSS) program Ver 22. *P* value of $p < 0.05$ was considered indicative of a statistically significant difference.

Result and Discussion:-

A total of (120) Otitis Media swabs were subjected for culture on different types of media, The result revealed that 33 (27.5%) Gram positive bacteria represents *S.aureus* (25) 21%, *S.epidermidis* (8) 7%, While 87 (72.5%) Gram negative bacteria represents *P.aeruginosa* (45) 37.5%, *A.baumannii* (16) 13%, *Proteus* sp (12) 10%, *E.coli* (8) 7%, *P.fluorescence* (6) 5%, and These results were shown in figure (1).

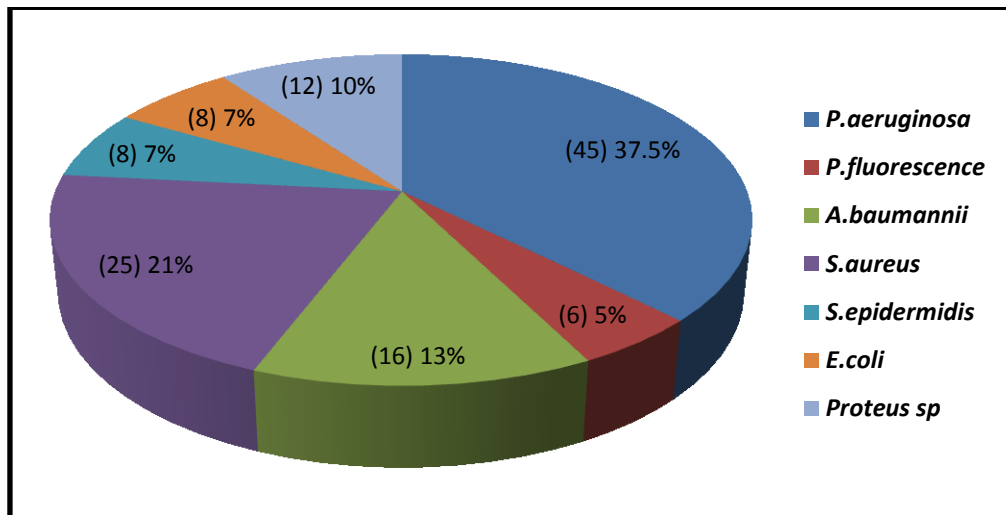


Figure (1): Frequency of bacterial isolates in Otitis Media (OM)

According to these results *P.aeruginosa* was considered to be the dominant bacteria isolated in this study, this result is agree with [16]. In every cases as often found of chronic otitis media include *P.aeruginosa* is the most frequently isolated infective pathogen [17]. the second most common pathogen *A.baumannii* represent (16) 13%, this result not agreed with [18], that found the *A.baumannii* was representing in (2.77%). While *proteus* sp represent (12) 10%, this result not consistent with another study on patients with otitis media, the *proteus* specie constitute 14.7%, while bacteria other than *proteus* sp represent 85.3% including *S.aureus* and *P. aeruginosa* [19]. Other Gram negative bacteria isolated from Otitis media with low percentage are *E. coli* (8) 7% and *P.fluorescence* (6) 5%. The difference in the pattern of microbiological isolates may be explained by differences in the geographical situation and population dynamics [20].

Gram positive bacterial isolates in this study included *S. aureus* (25) 21%,and *S. epidermidis* (8) 7%, this result is not match with [21] this may be due to the actuality that Staphylococci is a part of normal flora of ear canal and Eustachian tube . The result agreed with [22].

Antimicrobial activity of Olive leaves extract and apple cider vinegar:

The results of the experiment are show in figure (2&3), As demonstrated from these figures with use different concentration (200,400,600,800,1000)mg/ml on various pathogenic bacteria showed diverse levels of sensitivity toward aqueous and alcoholic olive leaves extracts and apple cider vinegar and their effect (zone of inhibition in mm) on different bacterial isolates.

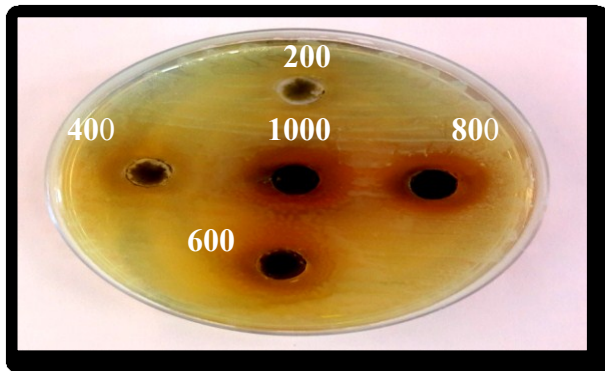


Figure (2): inhibition zone of olive leaves extract against *S.aureus*

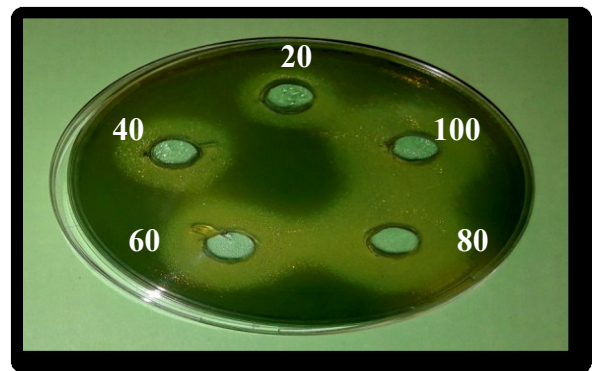


Figure (3): inhibition zone of apple cider vinegar against *P. aeruginosa*

The results in Figure (4), revealed that there is significant difference ($P<0.05$) among various concentrations of cold aqueous olive leaves extract against pathogenic bacteria. The cold aqueous extract of olive leaves gave high effect against *Proteus* sp range 10-28mm in all concentration, while moderate levels of inhibition against *P. aeruginosa*, *E.coli*, *A.baumannii* range 10-25mm, low level of inhibition on other bacteria.

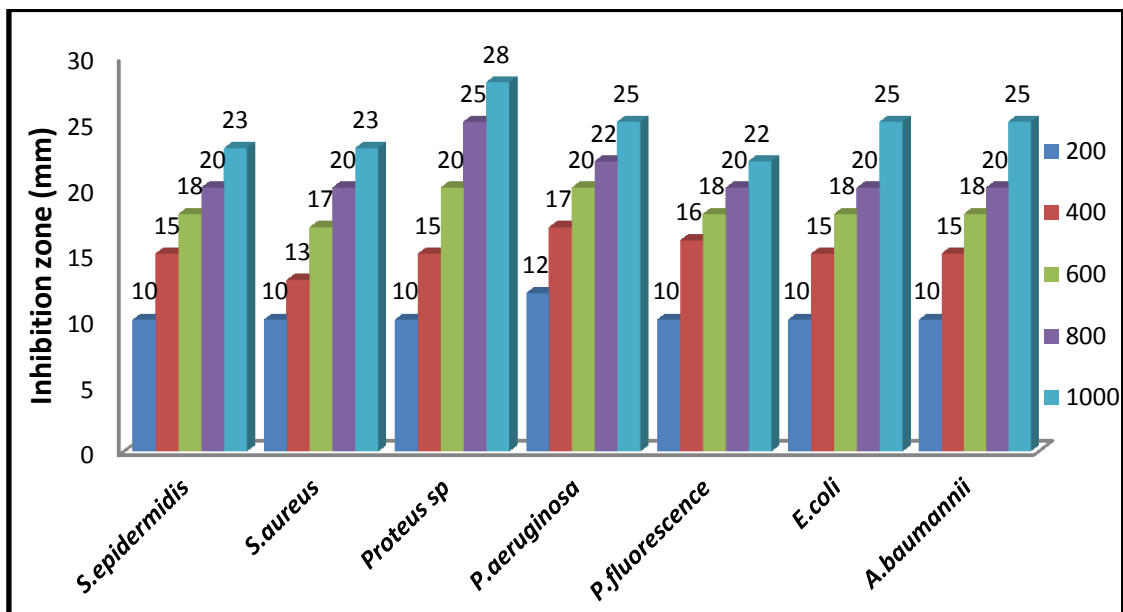


Figure (4): Antimicrobial activities profile (inhibition zone in millimeter) of different concentrations (200,400,600,800,1000)mg/ml from cold aqueous olive leaves extract against tested bacterial.

Boiled aqueous extract showed variable level of inhibition ranging from 10-30mm in all concentration, both *S.aureus* and *P.fluorescence* to high levels from inhibition range 14-28mm, and moderate levels range 10-23mm to word other bacteria, there is significant difference ($P<0.05$). as show in figure (5).

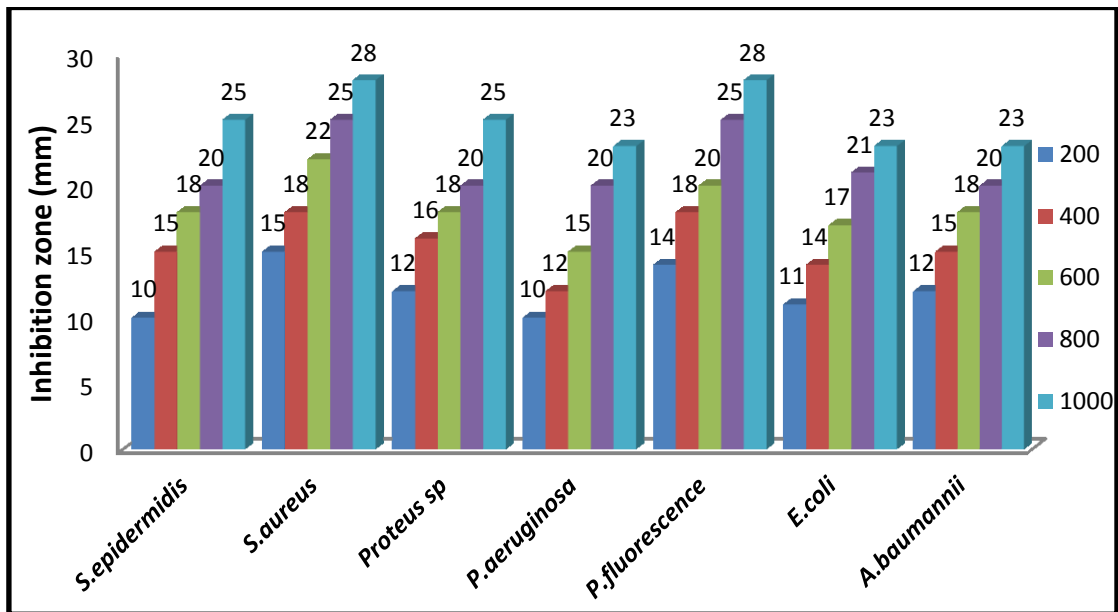


Figure (5): Antimicrobial activities profile (inhibition zone in millimeter) of different concentrations (200,400,600,800,1000mg/ml) from boiled aqueous olive leaves extract against tested bacterial.

The results indicated that the Boiled olive leaves extract are more effective than Cold olive leaves extract as show in figure (4,5). This result is agree with [23]. That the olive leaves contains phenols, and phenolic compounds are oxidized in Free radicals reactions and have the ability to inhibit microbial adsorption, Enzymes and proteins that transport the cytoskeleton.

On the other hand, the ethanolic extract of olive leaves gives better extraction than Cold & Boiled aqueous extract figure (6). show the significant difference ($P < 0.05$) between difference concentration ethanolic olive leaves extract against pathogenic bacteria. variety level of inhibition ranging from 10-30mm, that the *P. aeruginosa* & *Proteus sp* at high levels of inhibition range 15-30mm, while other bacteria with moderate levels of inhibition.

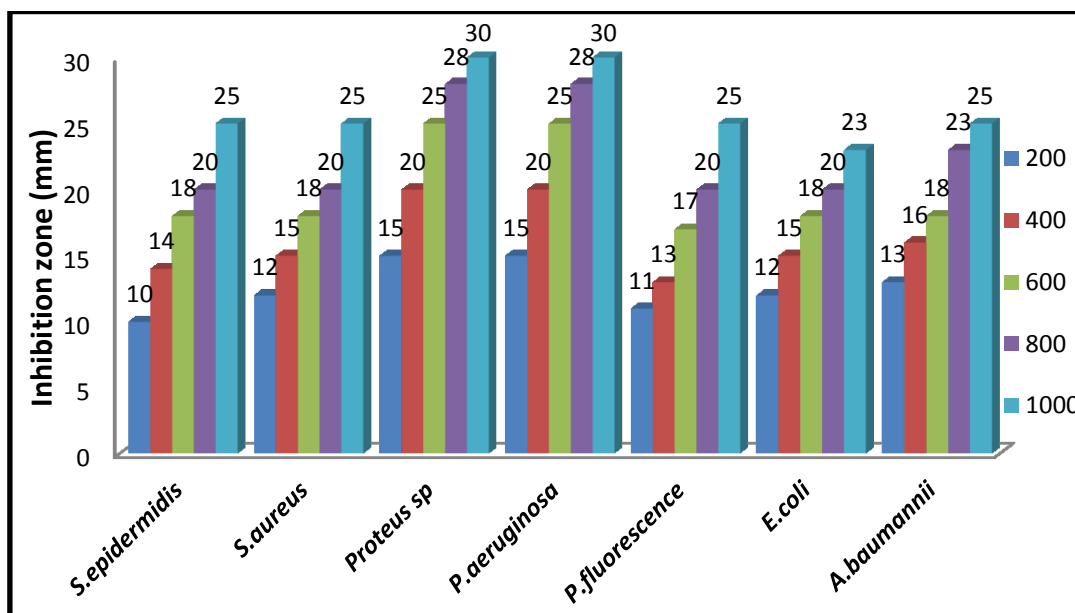


Figure (6): Antimicrobial activities profile (inhibition zone in millimeter) of different concentrations (200,400,600,800,1000)mg/ml from alcoholic olive leaves extract against tested bacterial.

The researchers in [24] have confirmed that the ethanol extracts of the tested plants have a higher biological effects than the aqueous extracts on the growth of microorganism. The results has resemblance between the other studies. about the antimicrobial activity of olive leaves extracts. phenolic compounds found extract have the antibacterial activity due to their ability to cause damage bacterial membrane and disrupt the cell wall peptidoglycan, which cause loss of structural integrity and leakage of intracellular cytoplasmic constituents such as protein, glutamate, potassium and phosphate [25]. [26] In their study found that the olive leaf extract have antibacterial activity to higher for the gram negative strains as compared to gram positive strains. Some researchers reported that the oleuropein which is included in these products has a set of pharmacological properties including antioxidant, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic and antiviral activities [27].

In comparison with Apple Cider Vinegar which showed highest antibacterial activity compared with Aqueous and Alcoholic olive life extract against tested bacteria with the significant difference ($P < 0.05$). The result about of antibacterial activity to apple cider vinegar they were studies, the results showed agar diffusion of vinegar that a high activity of apple cider vinegar against bacterial isolates. The maximum inhibition zone range from 15-35mm. Gram negative bacteria was observed inhibition zone against *E.coli*, *A.baumannii* 20-37mm, another bacteria include (*P.aeruginosa*, *P.fluorescence*) & *Proteus* sp the inhibition zone range 15-35mm & 15-30mm respectively. while observed the maximum inhibition zone of Gram positive bacteria 15-30mm, as show in figure (7).

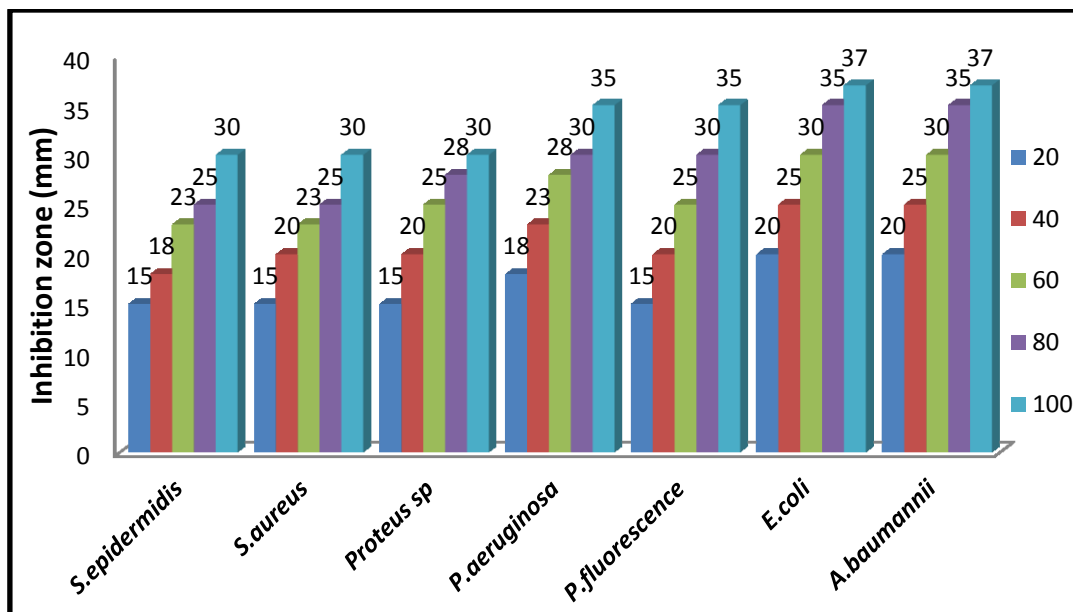


Figure (7): Antimicrobial activities profile (inhibition zone in millimeter) of different concentrations (20,40,60,80,100)ml/ml from of apple cider vinegar against tested bacterial

This result agreed with another study used various vinegar solutions concluding that 5% apple cider vinegar eradicated streptococcal biofilm successfully [28]. In another study is consistent with [29], That the Apple cider vinegar with high effect against gram positive and gram negative bacteria. Although, contain high amount of organic acids, mainly acetic acid which their antimicrobial effect was particularly weak. Other components such as ketonic compounds, phenolic compounds and neutral compounds may be key compounds in bio efficacies, Phenolic compounds include cresols and phenol well known to have antimicrobial property and the association between the structures and antimicrobial activities of substituted phenols were already reported [30]. The

antibacterial effect of organic acids on different types of pathogenic bacteria have proved by diversity of researchers [31].

Conclusions :

Alcoholic olive leaves extract have been high effect toward most bacterial isolates rather than Aqueous (cold & boiled) Olive leaves extract. In comparison Apple cider vinegar high effect than Olive leaves extract towards pathogenic bacteria.

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