



## Detection of the Inhibitory Effect of the Leaves, Seed and Fruits of *Cydonia oblonga* on some Gram Positive and Negative Bacteria

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### ABSTRACT

Seventy six swab samples were collected from Otitis media and stool samples from gastroenteritis cases, were cultured on different culture media, gram positive and gram negative bacteria were isolated and identified. The highest prevalence was *Staphylococcus aureus* (25%) while *Salmonella typhi* formed the lowest isolate rate (3.9%) other bacterial isolates varied between these two and comparison was done between effect of the air part of *Cydonia oblonga* (Quince) plant (leaves, seeds, fruits) to determine the highest effective part on the isolated bacteria. As the inhibitory effect of *Cydonia oblonga* seed, leaves and fruit alcoholic and aqueous extracts on the isolated bacteria were studied. Alcoholic seed extract showed the highest inhibitory activity on two bacterial species *Enterobacter aerogenes* and *Enterococcus faecalis* (25 and 22 mm) respectively followed by *Aeromona* sp. and *Pseudomonas aeruginosa* (13 and 11 mm) respectively while alcoholic leaves extract showed antibacterial effect on *Staphylococcus aureus* only (15 mm), alcoholic fruit extract showed its effect on *Enterococcus faecalis* (12 mm), *Klebsiella* sp. (11 mm), *Salmonella typhi* (10 mm), and *Pseudomonas aeruginosa* (9 mm). the aqueous seed extract showed an effect on *Enterococcus faecalis* (11 mm), while the aqueous leaves extract showed the highest effect on *Staphylococcus aureus* (20 mm), followed by *Bacillus subtilis* (10 mm), and the aqueous fruit extract showed the highest effect on *E.coli* (10 mm) while on *Enterococcus faecalis*, *Klebsiella* sp. and *Enterobacter aerogenes* was less (9 mm) for all of them. The (MIC) value of the effective extracts revealed that the highest value of alcoholic seed extract on *E.faecalis* was (25 mg/ml), while the least (MIC) was (0.39 mg/ml) for *Aeromona* sp. with the remaining MIC values ranging between the two.

**Keywords:** antibacterial activity, *Cydonia oblonga* (quince plant).

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### INTRODUCTION

Otitis media is a cavity filled with air located in petrous bone cavity. It is one of the most common infections which occur at all ages but is most common in young children. The pathogenesis of Otitis media is multifactorial, implicating interaction between the environment, pathogen, local anatomy, and host. Several risk factor have been identified including environmental tobacco smoke exposure, season of the year, respiratory tract infection, and the host immune response to the infections in the middle ear, day care, siblings bottle- feeding (Daly *et al.*, 2010; Uhari *et al.*, 1996).

Gastroenteritis is an infection or inflammation of the digestive system. most gastroenteritis are contagious, it is caused by a number of different micro-organisms including bacteria like (*Campylobacter*, *Salmonella*, *Shigella*) or caused by virus (*Rotavirus* And *Norovirus*) or parasites (*Giardia*, *Cryptosporidium*). It can also be caused by toxins produced by bacteria examples (*Staph.aureus*, *Enterobacter cloacae*, *Clostridium perfringenes*). the symptoms of infection including diarrhea, vomiting, stomach pain, fever (Meshram *et al.*, 2009; Sulayman and Shareef, 2007; Koneman *et al.*, 1997).

The development of bacterial resistance to antibiotics has necessitated the studies for new antibacterial agents. It is well known that infectious diseases account for high proportion of health problems, especially in developing countries. Microorganisms have developed resistance to many antibiotics and this property has created expansion of clinical problems in treatment of infectious diseases (Davis, 1994).

The increased resistance to the commercial antibiotics due to the incorrect and miss use of antibiotics treatment of infectious diseases. this case lead the scientists to search for new antimicrobial substances from different sources, such as medicinal plants which have been used for centuries as remedies for human diseases due to its high contents of active components of therapeutic value. The medicinal plant constitute an important part of human food. In addition to its flavoring and preservative effect. In addition to its medicinal uses (Adu, 2009; Betoni *et al.*, 2006).

*Cydonia oblonga* (Quines plant) is one of the medicinal plants used in alternative medicine for its effect, it is used for intestinal infections, and anti-injury etc.

The taxonomy of *Cydonia oblonga* Miller (Quince), kingdom Plantae, order Rosales, family Rosaceae, genus *Cydonia* (Mill), species *C.oblonga* (Potter *et al.*, 2007; Townsend and Guest, 1966).

Quince is deciduous shrub or small tree leaves were entire, petiolate. The flowers were solitary, terminal usually rather large petals. fruit have many seeds. These plants originate from central Asia and distributed in Iraq, Syria and Turkey (Fattuch *et al.*, 2007; Townsend *et al.*, 1980; Townsend and Guest, 1966).

The fruits subglobose or pyriform, 6-10 cm or larger, yellowish, hard and sour, however the fruit becomes palatable on cooking and owing to its pleasant aroma has long been used in many countries for marmalade, pies, jellies. The fruit is astringent expectorant, as atonic for cardiac patients.

The seeds are highly mucilaginous, and used as a demulcent, expectorant and tonic effect has been widely known since antiquity and are used till this day in Iraq popular remedies for coughs, bronchial and other complaints, intestinal infections, antidiarrheal, anti-gastrointestinal, antivomiting (Jber, 2009; Chackravarty, 1976). The useful effect of the fruit, seeds and leaves of quines to human was due to its content of many vitamins like vitamin A and B in addition to other constituents including sulphat, phosphorus calcium, magnesium, choride, potassium and fibers, pectine, acids and many active substances like phenols and glycosides (Rop *et al.*, 2011; Townsed *et al.*, 1980; Chakravarty, 1976).

The quince fruit is known for its medicinal properties as antioxidant, antimicrobial and anti-injury which have been recently attributed to its active constituent. Quince tree leaves, have also a role in removing free radicals and was considered as anti-hemolytic factors (Osman *et al.*, 2010).

The aim of the study was to investigate the antibacterial effect of seeds, leaves and fruit extracts of *Cydonia oblonga* and to compare the anti-effects against the isolated bacteria.

## MATERIALS AND METHODS

### 1- Materials:

#### A- Isolation and Identification of Bacteria:

##### A-1- The Specimens:

Seventy six samples were collected from patient with Otitis media and gastroinritis their ages ranged between (2-40) years from (ENT) and medical unites in Ibn Al- Atheer and Al- Salam hospitals. (51) Ear swab's samples were collected after clinical examination of patients, swabs were transferred to modified stuart transport medium while (25) stool samples were collected in clean container then cultured on primary culture media.

**A-2- Cultural Media:****A-2-1- Commercial Media:**

The culture media were prepared according to the instructions of company and the (pH) was adjusted and then sterilized by autoclave.

Culture media includes:

Nutrient agar, MacConkey agar, nutrient broth, blood agar, DNase agar, citrate agar, sugar fermentation medium, Mannitol salt agar, Salmonella- Shigella agar, Muller-Hinton agar.

**A-2-2- Prepared Culture Media:**

Media were prepared from their components depending on (Difco, 1998; Koneman *et al.*, 1997) such as: Blood agar, Glucose- phosphate pepton water, Starch agar, Mitis salivarius agar base (M 259).

**A-3- Stains and Solutions:**

Prepared according to (Macfaddin, 1980; Koneman *et al.*, 1997) including gram's stain, catalase reagent (3%), oxidase test reagent, kovac's reagent, methyl red reagent, voges- proskauer reagent.

**2- Methods:****- Cultivation on Primary Media:**

The samples were cultured on, MacConkey agar and blood agar, incubated at (37)°C for (18-24) hours and incubated blood agar was incubated in the presence of 5-10 % of CO<sub>2</sub>.

**- Identification:**

It was done depending on colonial characteristics on culture media then smears were prepared and stained with gram stain and examined microscopically for the shape arrangement of cells and their reaction with gram stain.

**- Biochemical Tests:**

Differential and selective media were used to identify some of the pathogenic bacteria, then oxidase test, catalase test, coagulase test, DNase test, IMViC reaction, sugar fermentation test, starch hydrolysis tests were performed according to (Tang and Straton, 2006; Koneman *et al.*, 1997).

**B- Preparation of Plant Extracts:**

Fresh fruits, leaves and seeds of quince (*Cydonia oblonga Mill.*) were collected from gardens of Mosul city. The parts were washed and dried at room temperature.

**- Preparation of aqueous extracts:**

Fruits and leaves were cut in to smaller parts, then (40) gms from each of fruit, leaves and seeds were added to a flask containing (160) ml of D.W (1: 4) weight: volume, were grinderd using blender in an ice bath for (60) minutes to disrupt the walls of plant. Kept at (4) °C for (24) hours for macerating, then the mixture was filtered through many layers of gauz, then filtrated again through (Whatmann paper No.1) under vaccume then extract were obtained (Riose, *et al.*, 1987) which was dried by lyphilization in lyophilizer produced by Edwards company then samples were kept in glass vials and stored at (-20) °C (Riose *et al.*, 1987; El- Astal *et al.*, 2005).

**- Preparation of ethanolic extracts:**

Fruit and leaves were divided in to smaller parts, (20) gm each of the fruit, leaves and seeds were crushed in (200) ml of ethanol (95) % in an ice bath, the solution was left in refrigerator for (24) hours then filtered using many layers of gauze, after that alcohol was evaporated using rotary vaccume evaporator, (Electrothermal English company) at (40) °C. a thick layer of extract which was left kept in glass vials at (-20) °C (Adomi, 2006; El- Astal *et al.*, 2005).

**- Sterilization of aqueous and ethanolic extract:**

One gm of dried plant extract was dissolved in (5) ml of D.W to prepare an extract concentration (200) mg/ml as standard concentration which was sterilized by filtration using Millipore filter (0.22 μ). serial dilutions was prepared from the stock solution. While when prepared the ethanolic extract one gm of plant extract was dissolved in (5) ml of dimethyl sulfoxide to

prepare concentration of (200) mg/ml as a stock solutions which was sterilized by pasteurization at (62) °C for 30 Sec.

**- Determination of the antimicrobial activity:**

The disk diffusion method by (Bauer *et al.*, 1966) was used, the bacterial suspensions were prepared then the bacterial concentrations were fixed at  $1.5 \times 10^8$  cell/ml by comparison with MacFarland standard No. 1, then a sterile cotton swab were impregnated with the broth culture which was rotated several times with firm pressure on the inside wall of the tube to remove excess fluid, then dried surface of the Mueller Hinton agar plate were seeded by streaking the swab three times over the entire agar surface by rotating the plate 60° after each streak, then filter paper disc impregnated with a stock of aqueous extracts of each (seeds, fruits and leaves) were distributed on surface of plate and disc soaked with ethanolic extract of each (seed, fruit and leaves) on another Mueller Hinton agar plate by using sterile forceps, and plates were incubated at 35°C - 37°C for (16-18) hr. the antimicrobial activity was determined by observing the diameter of inhibitory zone around the disks (Parija, 2007; Vandepitte *et al.*, 2003).

**- Determination of the minimum inhibitory concentration of the plant extracts (MIC):**

Serial dilutions of plant extracts were prepared in (DMSO) for ethanolic extract and in distilled water for aqueous extract (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195) mg/ml then (0.1) ml of each concentration was added to (9.8) ml of sterile nutrient broth and (0.1) ml of standard bacterial suspension ( $1.5 \times 10^8$ ) cells/ml was added to all tubes then incubated at 37 °C for 24 hr.

The tubes were examined for turbidity which was regarded as an indicator of the bacterial growth. The lowest concentration of extracts which inhibits the bacterial growth and was detected by lack of visual turbidity, was regarded as the MIC (Rios and Recio, 2005; Koneman *et al.*, 1997).

## RESULTS AND DISCUSSION

During the period of study (76) samples were collected from patient infected with Otitis media and gastroenteritis including ear swab and stool samples. Table (1) showed the percent of isolation of each bacterial species, *Staph.aureus* and *Bacillus subtilis* formed the highest isolates (25 %) and (18.4 %) respectively, while *Salmonella typhi* was the lowest (3.9 %), the other bacterial isolates ranged between them. Our results showed predominance of *Staph.aureus* in patient infected with Otitis media which accounts for (25 %) these results are similar to study of (Jwad, 2011) who isolate *Staph.aureus* at ratio of (24.6 %) and also to the result of (Al- Maadidi, 1999) who isolate it at ratio of 23.4 % (Al-Faris *et al.*, 1998) and (Khalil, 1980) confirmed that *Staph.aureus* constitute the highest isolation rate between other bacterial causes of infection.

The present study showed that *B.subtilis* was isolated from Otitis media (18.4 %). *Bacillus* sp. considered normal flora of external auditory canal and skin and also it is present in air as contaminant and hence isolated at a high rate (Al- Madidi, 1999). also isolate this bacterium in the a second class from samples of the control group. its isolation may be a results of contamination of otoscope.

*E.faecalis* and *E.aerogenes* constitute the highest rate of isolation from stool (6.57 %) while *Salmonella typhi* was isolated at a lower rate (3.9 %). Gastroenteritis was caused by many species of bacteria which was isolated at from cases and cause inflammatory changes through their invasion and production of enterotoxins in the intestine leading to diarrhea (Meshram *et al.*, 2009; Winn *et al.*, 2006).

**Table 1: The percentage of isolated bacterial species**

Isolated bacterial species	Source	Number	Percentage %
<i>Staphylococcus aureus</i>	O.m	19	25
<i>Bacillus subtilis</i>	O.m	14	18.42
<i>Pseudomonas aeruginosa</i>	O.m	9	11.84
<i>E.coli</i>	O.m, stool	7	9.21
<i>Klebsiella sp.</i>	o.m	6	7.89
<i>Enterococcus faecalis</i>	stool	5	6.57
<i>Enterobacter aerogenes</i>	stool	5	6.57
<i>Morganella morganii</i>	stool	4	5.29
<i>Aeromonas sp.</i>	O.m, stool	4	5.27
<i>Salmonella typhi</i>	stool	3	3.94
		<b>76</b>	<b>100%</b>

O.m. = Otitis media

### The antibacterial effect of the quines extract:

The results showed that the alcoholic extract of quinus plant tend to inhibit the growth of bacteria compared with aqueous extract, (Table 2) showed the antibacterial activity of quinus extracts against bacteria. The results showed that *C.oblonga* (quince) seeds ethanolic extract showed high antibacterial effect against *E.aerogenes* and *E.faecalis* (Fig. 1 and 2), and lower effect on *Aeromonas sp.* and the extract showed low effect on *Pseudomonas aeruginosa*. while no effect had been shown by the aqueous seeds extract against all the species of bacteria except *E.faecalis* which showed a clear effect as the inhibitory zone (11 mm).

**Table 2: The antibacterial effect of the aqueous and ethanolic extract of quines plant on isolated bacteria**

Bacterial species	Plant extract of quines plant					
	ethanolic seeds extract	aqueous seeds extract	ethanolic leaves extract	aqueous leaves extract	ethanolic fruit extract	aqueous fruit extract
<i>Staph.aureus</i>	-	-	15	20	-	-
<i>E.faecalis</i>	22	11	-	-	12	9
<i>Pseudo.aeruginosa</i>	11	-	-	-	9	-
<i>Aeromonas sp.</i>	13	-	-	-	-	-
<i>E.coli</i>	-	-	-	-	-	10
<i>Morganella morganii</i>	-	-	-	-	-	-
<i>Salmonella typhi</i>	-	-	-	-	10	-
<i>Klebsiella sp.</i>	-	-	-	-	11	9
<i>Bacillus subtilis</i>	-	-	-	10	-	-
<i>Enterobacter aerogenes</i>	25	-	-	-	-	9

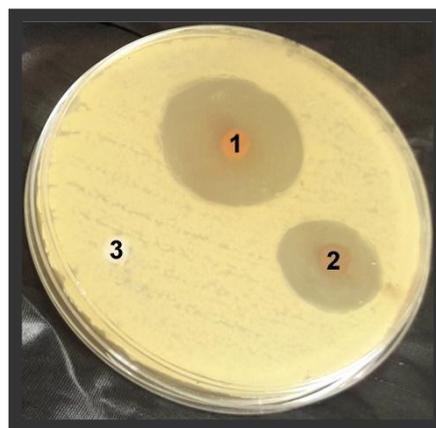
(- = no activity)

The aqueous leaves extract showed high effect on *Staph.aureus* 20 mm Fig. (3), while on *Bacillus subtilis* it was (10 mm) ethanolic leaves extract don't showed effect on bacteria except on *Staph.aureus* which give inhibitory zone of 15 mm Fig. (4). Ethanolic fruit extract showed had high effect on *E.faecalis* (12 mm), while low inhibitory effect on other species of bacteria. the aqueous fruit extract showed a very low activity on *E.faecalis*, *Klebsiella sp.* and *E.aerogenes*, (9 mm), while the inhibitory zone on *E.coli* (10 mm) from this results the antibacterial effect depends primarily on the type of solvent used for extraction it was observed that the ethanolic extract of

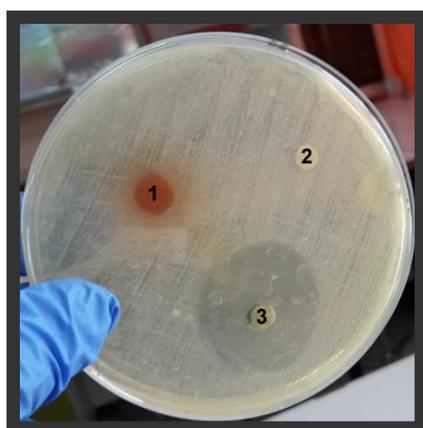
*C.oblonga* seeds was the most effective against tested bacterial however the aqueous extract showed insufficient effect and most species of bacteria were resistant to it. This results was similar to the study of (Cloch *et al.*, 2017; Alizadeh *et al.*, 2013).



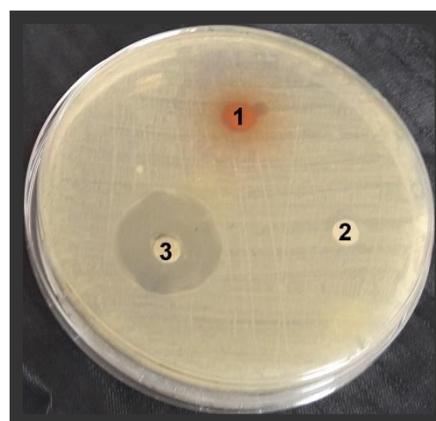
**Fig. 1: Antibacterial activity of Quince seeds alcoholic extract on *E.aerogenes***  
(1= seeds, 2= fruit, 3= leaves)



**Fig. 2: Antibacterial activity of Quince seeds and fruit alcoholic extract on *E.faecalis***  
(1= seeds, 2= fruit, 3= leaves)



**Fig. 3: Antibacterial activity of Quince aqueous leaves extract on *Staph.aureus***  
(1= seeds, 2= fruit, 3= leaves)



**Fig. 4: Antibacterial activity of alcoholic leaves extract on *Staph.aureus***  
(1= seeds, 2= fruit, 3= leaves)

The study of (Shahata *et al.*, 2011) showed that the effect of ethanolic seeds toward *Staph.aureus*, *Streptococcus pneumoniae*, *K.pneumoniae* and *Pseudo.aeruginosa* gave different diameters of inhibition zone at different concentration of extracts used.

The inhibitory effect of quines seeds was referred to its contents of active substances like glycosides, phenols, tannines, ratenges, comarens and oily materials (Shahata *et al.*, 2011) and the study of (Alizadeh *et al.*, 2013) showed the main aromatic compounds of *Cydonia oblonga* were five including caryophyllene, Eucalyptol, camphor, morphinan-3, 6-± diol, 7, 8- did hydro-4, 5-± epoxy- 17- methyl-, 6- acetate and trimethyl hydrazine which were identified by GC- MS method. The dissimilarity of the effect of extract toward the bacterial isolates was due to dissimilarity of the methods used for extraction and differential content of active materials.

The result of our study were similar to that of (Shahata *et al.*, 2011) with respect to the effect of alcoholic seeds extract on *Pseudomonas aeruginosa* was moderate (14 mm), and our results were similar to the study of (Alizadeh *et al.*, 2013) the *Enterobacteriaceae* were sensitive to quenes extract, while (Cloch *et al.*, 2017) showed that no inhibitory effect had been detected using the alcoholic leaves extract against *E.coli*, *Pseudomonas*, and *Bacillus*, these results were similar to our results, while the aqueous leaves extract inhibited the growth of *E.coli*, *Pseudomonas*, and *Bacillus*, our results inhibited *Bacillus subtilis* and *Staph.aureus* only.

Our results was consistent with that of (Al-Abacee, 2016) with respect to the effect on *Staph.aureus*. The effect of leaves extracts was due to presence of active constituents as tannines and pectins. The seeds extract showed better activity than leaves extract because the content of active materials were more than leaves extract. The aqueous fruit extract showed the effect on *E.coli* in our result is similar to (Cloch *et al.*, 2017).

The extracts obtained from different parts of the same plant showed various activity against bacteria under study the aqueus leaves extracts was characterized by stronger effect on bacteria than fruit extracts. The study of (Silva *et al.*, 2005) showed different polyphenol composition of individual morphological parts of quince. The leaves extract contain flavonol glycosides, derivative of quercetin and phenolic acid which are present at low concentration in fruit extract.

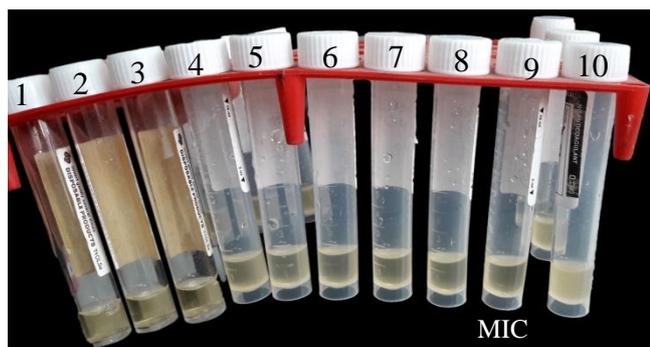
The composition of the various parts of the same plant is directly related to the degree of its maturity and its exposure to environmental conditions. The content of poly phenolic compounds changes during growth and ripening of quince, which may affect its antimicrobial properties (Wojdylo, 2011).

Table (3) showed the minimum inhibitory concentration (MIC) for *Cydonia oblonga* Miller extract toward the tested bacterial species. Its appeared that the MIC of *E.aerogenes* equal to (0.78) mg\ml for ethanolic seeds, Fig. (5), while MIC of *Staph.aureus* equal to (3.125) mg\ml for equeos leaves Fig. (6) and (6.25) mg\ml for ethanolic leaves extract, while *Aeromonas sp.* equal to (0.39) mg\ml for ethanolic seeds, and *E.faecalis* was (25) mg\ml for the same extract.

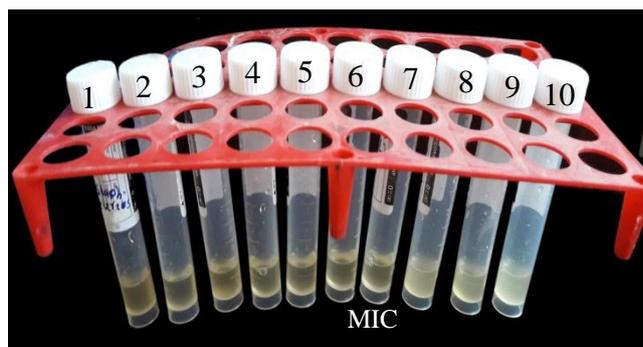
**Table 3: Minimum inhibitory concentration (MIC) of quines plant extract on tested bacteria**

Bacterial species tested	Quines extract		
	ethanolic seeds extract	aqueous leaves extract	ethanolic leaves extract
<i>E.aerogenes</i>	0.78 mg\ml	-	-
<i>Staph.aureus</i>	-	3.125 mg\ml	6.25 mg\ml
<i>Aeromonas sp.</i>	0.39 mg\ml	-	-
<i>E.faecalis</i>	25 mg\ml	-	-

(- = no activity)



**Fig. 5: MIC of *E.aerogens* (MIC= 0.78 mg/ml)**



**Fig. 6: MIC of *Staph.aureus* (MIC= 3.125 mg/ml)**

The main components responsible for the antimicrobial properties of plant are poly phenolic compounds, the mode of effect of the active materials were by inhibitory the enzymes (phospholipase oxygenase) through binding with hydrosulfide groups and stops the activation of proteins (Al-Hadidi *et al.*, 2013; Cowan, 1999; Kim *et al.*, 1995).

Tannins protect a plant against the effects of microorganisms by the formation of complexes with proteins, while terpenes interfere with the incorporation of the lipophilic compounds in the bacterial cell membrane (Mendoza *et al.*, 1997).

The differences of the antibacterial activity compared with other studies may be due to many factors including local, seasonal, climatic and the experimental factors.

### CONCLUSION

The extract obtained from different parts of the same plant exhibited various activity against studied bacteria, and the ethanolic seeds of quines plant showed the highest effect against the bacterial species compared with aqueous extract, and the equeus leaves extracts showed better antibacterial activity against *Staph.aureus* and the effect of seeds and leaves extracts was the more effective.

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## التحري عن الفعالية التثبيطية لأوراق وثمار وبذور نبات السفرجل في بعض أنواع البكتريا الموجبة والسالبة لصبغة كرام

### الملخص

تم جمع 76 مسحة من المصابين بالتهاب الاذن الوسطى وعينات خروج من المصابين بالتهاب الامعاء، لقيحت على الاوساط الزرعية وعزل منها عدد من الجراثيم الموجبة والسالبة لصبغة كرام، سجلت جرثومة *Staphylococcus aureus* اعلى نسبة عزل بلغت (25%) فيما عزلت *Salmonella typhi* بأقل نسبة بلغت (3.9%)، وتراوحت نسب بقية الجراثيم بينهما. قورن بين فعالية الاجزاء الثلاثة الهوائية التابعة لنبات السفرجل (أوراق، بذور، ثمار) وتم تحديد الجزء الأكثر فعالية في التأثير على الجراثيم المعزولة.

تم دراسة التأثير التثبيطي لمستخلصات بذور وأوراق وثمار السفرجل المائية والكحولية وتأثيره على جميع الجراثيم المعزولة واطهر المستخلص الكحولي لبذور السفرجل اعلى فعالية تثبيطية على الجرثومتين *Enterobacter aerogenes* و *Enterococcus faecalis* (25 و 22) ملم على التوالي تلتها *Aeromonas sp.* و *Pseudomonas aeruginosa* (13 و 11) ملم على التوالي فيما اظهر مستخلص الاوراق الكحولي تأثيرا في جرثومة *Staphylococcus aureus* (15 ملم)، واطهر مستخلص الثمار الكحولي تأثيرا في *Enterococcus faecalis* (12 ملم) و *Klebsiella Sp.* (11 ملم)، و *Salmonella typhi* (11 ملم) و *Pseudomonas aeruginosa* (9 ملم)، واطهرت المستخلصات المائية للبذور تأثيرا على *Enterococcus faecalis* (11 ملم) في حين اظهر مستخلص الاوراق المائي اعلى فعالية على جرثومة *Staphylococcus aureus* (20 ملم) تلتها *Bacillus subtilis* (10 ملم)، اما مستخلص الثمار المائي فأظهرت *E.coli* أعلى فعالية (10 ملم)، اما *Enterococcus faecalis* و *Klebsiella sp.* و *Enterobacter aerogenes* فأظهرت فعالية اقل (9 ملم) لكل منها. وتم تقدير التركيز المثبط (MIC) للمستخلصات المؤثرة على الأنواع الجرثومية حيث بلغت قيمة (MIC) للمستخلص الكحولي لبذور السفرجل على جرثومة *E.faecalis* (25 ملغم/مل) واقل قيمة (0.39 ملغم/مل) على جرثومة *Aeromonas sp.* وتراوحت قيم بقية المستخلصات بينهما.