AJPS, 2013, Vol. 13, No.2

The antibacterial activity of *Trigonella foenum-graceum* extracts against bacteria that causes Otitis media in children.

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Abstract:

Two hundred fifty ear swab samples were collected from patient with Otitis media in children between 6-12 years old in the central children hospital in Baghdad for a period between July 2011 to December 2011.

The diagnosis of the isolates showed that 150 samples were positive to isolate bacterial culture indicate the presence of *Proteus* spp. 48(32%) then *Staphylococcus* spp. 45(30%), *Pseudomonas* spp. 40(26.6%) then *Escherichia coli* 17 (11.3%). The susceptibility of the isolates to antimicrobial agents were tested on seven antimicrobial agents by using disc diffusion assay.

The antimicrobial agents were ampicilin, Gentamycin, cephalothin, rifampin, tetracyclin, streptomycin, amikaci. All the isolates resist the streptomycin, tetracycline, rifampin,cephalothin and ampicillin.

The alcoholic and water extract of *Trigonela foenum graceum* shows activity against the four bacterial genuses. The (MIC) values for alcohol extract were (25mg/ml for *Staphylococcus spp*, 12.5mg/ml for *E. coli*, (25mg/ml) for *Proteus* spp. and 12.5 mg/ml for *pseudomonas* spp. The (MIC) values for the water extract were (25mg/ml) for *Staphylococcus spp.*,(12.5mg/ml)for *E.coli* and (25mg/ml) for both *Proteus spp.* and *Pseudomonas spp.*

Keywords*: Trigonella foenum graceum, antibacterial, otitis media infection.*

الخلاصة:

جمعت مائتان وخمسون عينة لمسحات من مرضى التهاب الاذن الوسطى من الاطفال بين عمر 6-12 سنه في مستشفى الطفل المركزي في بغداد للفتره من تموز 2011 لغاية كانون الاول 2011، وبعد تشخيص العينات وجد ان 150 عينه كانت موجبه وهي على التوالي *Proteus* spp. 48(32%), *Staphylococcus* 45(30%), *Pseudomonas* spp.40(26.6%), E .coli 17(11.3%),

أختبرت حساسية البكتريا ضد 7 من المضادات الحيوية بأستعمال طريقة الاقراص واستعملت المضادات وهي: امبيسيلين ,جنتامايسين, سيفالوثين, ريفامبين, تيتراسايكلين, ستربتومايسين و اميكاسين. وكانت كل العزلات مقاومه للستربتومايسين ,تيتراسايكلين, ريفامبين, سيفالوثين والامبسلين.

اختبرت الخلاصة الكحولية والمائية للحلبة وكان تأثيرها بنسب متوازنه على البكتريا قيد الدراسة، وتم تحديد التأثير المثبط الادنى (MIC) للمستخلص الكحولي على البكتريا اعلاه حيث كانت 25 ملغرام لكل مل من *Staphylococci* spp. و(12.5) ملغرام لكل مل من *E.coli* (25) *ملغرام لكل مل من Proteus spp و (12.5) ملغرام لكل مل من pseudomonas spp.* , ومن ثم تحديد التركيز المثبط الادنى (MIC) للمستخلص المائي على البكتريا اعلاه حيث كانت (25) ملغرام لكل مل من *staphylococcus spp.* و *(12.5)* ملغرام لكل مل من *E.coli* و *25 ملغرام لكل مل من Proteus spp.* و *Pseudomonas spp.*

مفاتيح الكلمات: الحلبة، التأثير التثبيط، التهاب الأذن الوسطى .

Introduction:

Trigonella foenum-graceum is an annual herb [1]. *Trigonella foenum-graceum* belongs to the family Fabaceae was selected to assess its antibacterial activity. Plant are hardy to about -15C [2] .It cultivated in India, Africa, Egypt, Morocco, and occasionally England [3]. Various medicinal properties like anti-cholestrolemic, antiinflamatory, antitumor, cardiotonic, carminative, demulcent, diuretic, emollient, expectorant, febrifuge, galactogogue, hypoglycemic, hypotensive and laxative have been attributed to this plant in the traditional system of Indian medicine. Fenugreek is much used in herbal medicine, especially in North Africa, the Middle East and India.It has wide range in midicinal applications. The seeds are give to convalescents. Researches has Shown that the seeds can inhibit cancer of liver, lower blood cholesterol levels and also have an antidiabetic effect [2].

It contains lecithin and choline that helps to dissolve cholesterol and fatty substances, minerals, vitamine B. Complex, iron, Phosphates, PABA (Para-Amino Benzoic Acid), and vitamins A and D. It also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system [4]. Otitis media is an infection or inflammation of the middle ear.

This inflammation often begins when infections that cause sore throats, colds, or other respiratory or breathing problems spread to the middle ear. These can be viral or bacterial infections. Seventy-five percent of children experience at least one episode of otitis media by their third birthday. Almost half of these children will have three or more ear infections during their first 3 years. It is estimated that medical costs and lost wages because of Otitis media amount to $5 billion a year in the United States. Although Otitis media is primarily a disease of infants and young children, it can also affect adults [5]. In recent years, there has been gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries, because plant derived drugs have been reported to be safe and without side effects[6].

Nowadays multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drug commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hyper-sensitivity, immune suppression and allergic reactions. Thus, there is a pressing need for new plants-based drugs [7,8]. The aim of the current study is to isolate the bacteria that cause otitis media infection and evaluate the antimicrobial activity of the alcohol and water extracts of *Trigonella foenum-graceum.*

Materials and Methods:

Collection of samples:

The medicinal plant used for the experiment was *Trigonella foenum graceum* authenticated duplicate pressed specimen of reference material obtained from (national herbarium of Iraq Botany directorate of Abu-Ghraib). I used the seeds part of the plant.

Antimicrobial tests:

Antimicrobial activities were tested by the disc diffusion method. All bacterial pathogens isolated were subsequently evaluated for suscep-tibility to seven antibiotics.

Extraction procedure:

Two extracts of *Trigonella foenum graceum* (water and alcohol) were prepared by taking (50gm) from the plant in known solvent 250 ml to get the desired

concentration, seeds of the plant were crushed by using pestle and mortar, after that weighted amount of the plant seeds dissolved in the solvent (ethanol, water) for 24 hours with intermittent shaking each extracted material was filtered through whatman filter paper number 1 and centrifuged1500 r.p.m for 15 min. And the supernatant used for antimicrobial testing [9].

Determination of the inhibition zones:

The freshly prepared inoculums were swabbed all over the surface of Mueller Hinton agar confluent, using sterile cotton swab. Five wells of 8 mm diameter were bored in the medium by sterile cork-borer and were labeled properly and prepare 3 concentration of (alcohol, water) extract, the weighted amounts is 200mg/ml, 400mg/ml, 600mg/ml, filled the wells with each solution concentration subsequently and leave it overnight incubation at 37c. The disc diffusion results were compared with the triple concentration of each extract, and record the zone of inhibition differences.

Minimum inhibitory concentration:

The minimum inhibitory concentration MIC was measured for the effect of (alcohol, water) extracts, we made two fold serial dilution for the alcohol extract by taking six test tubes contain 1 ml of nutrient broth the first one is control, we take 1 ml from the original alcohol extract and mix it with the second tube and mix well and take 1 ml from the same tube to the third tube and mixed well and so on to the sixth tube take 1 ml from the bacterial growth suspension to each tube ,and we subculture the last three tubes into the plates and incubate overnight at 37°C, the lowest concentration of alcohol extract that gives no growth this is known as MIC[10].

The statistical analysis:

I used the SAS statistical system[11] .

Results:

As can clearly seen from table-1, figure (1, 2, 3, 4) the 7 antibiotic discs that used in the disc diffusion test (sensitivity test) found to be effective against all tested bacteria used in the study in different level showing inhibition zone of 9 to 21 mm. . Table-1 shown the antimicrobial activities of some standard antibiotics by disc diffusion test after applying SAS statistical analysis system, fig (1, 2, 3, 4) showing inhibition zones formed by the standard 7 antibiotics on the 4 tested bacteria. Figure-5 histogram showing the sensitivity test by wells of both alcohol and water seeds extracts on the test bacteria. Figure-6 histogram showing the sensitivity test by wells of both alcohol and water seeds extracts on the test bacteria.

Table -1: Antibacterial activities of some standard antibiotics by disc diffusion test.

Bacteria	Inhibition zone formed by antibiotics in millimeters (mm)							Bacterial inhibition zone mean
	Ampicilin	Gentamicin	Cephalothin	Rifampin	Tetracycline	Streptomycin	Amikacin	
E coli	0.00	8.66	0.00	10.66	0.00	0.00	17.66	5.286
Staphylococci	0.00	19.66	0.00	0.00	26.00	0.00	21.66	9.619
Sseudomonas	0.00	17.66	0.00	0.00	0.00	0.00	18.66	5.095
Proteus	0.00	17.66	0.00	0.00	0.00	0.00	19.66	5.286
Inhibition zones mean	0.00	15.833	0.00	2.66	9.166	0.00	19.25	
LSD Bacteria (0.05)	0.233							
LSD Inhibition zone (0.05)	0.309							
LSD (0.05) Interaction	0.729							

Figure-1: E coli

Figure-2: Staphylococci

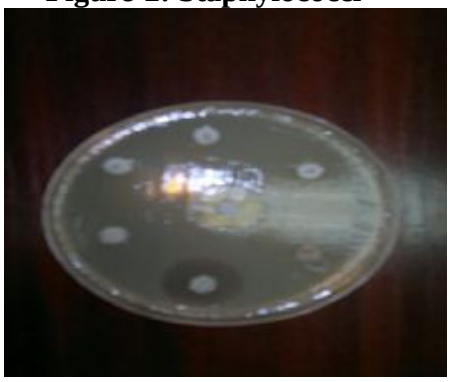
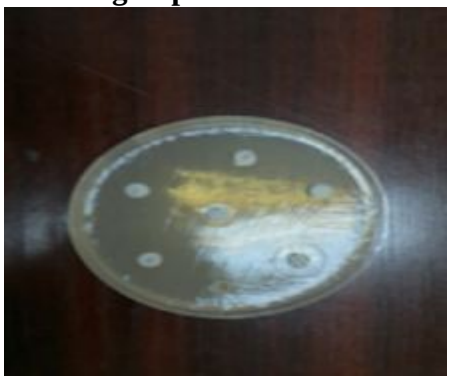


Figure-3: Proteus

Fig-4: pseudomonas



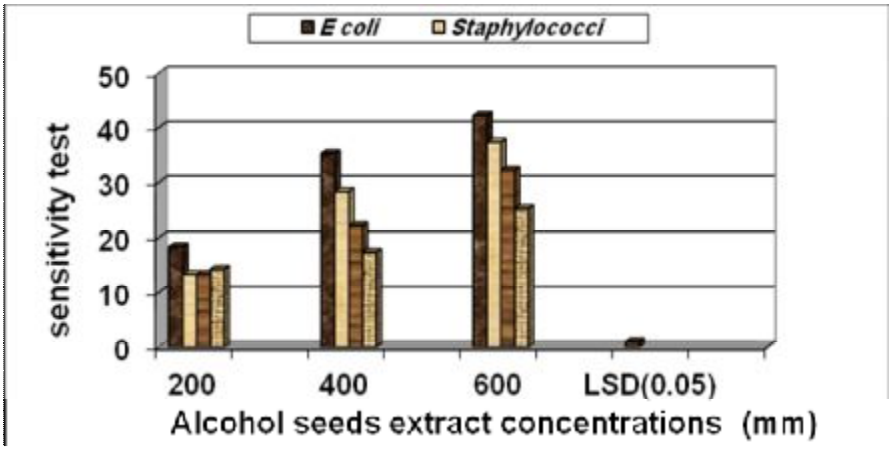


Figure-5: Histogram showing the sensitivity test by wells of both alcohol and water seeds extracts on the test bacteria.

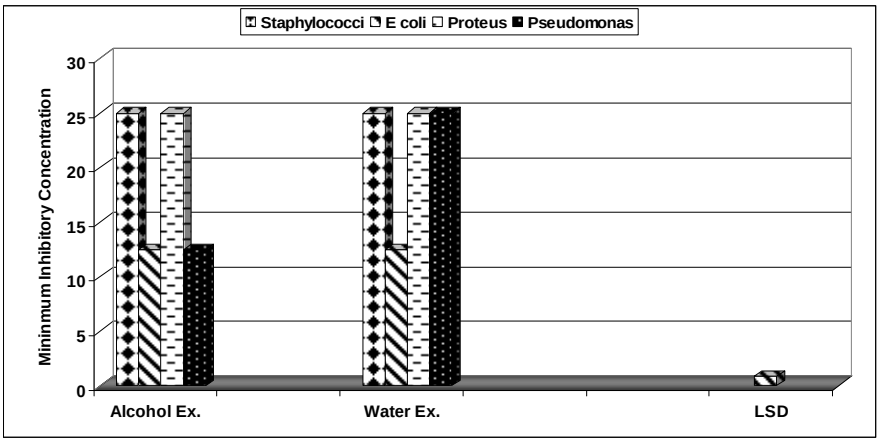


Figure-6: Histogram showing the (MIC) minimum inhibitory concentration of both the water and alcohol seeds extracts on the test bacteria.

Discussion:

The activity of the plant extracts against bacteria have been studied for years ago, but in more intensified way during the last three decades.

During this period, numerous antimicrobial screening evaluations has been published based on the traditional use of Chinese, African and Asian plant based drug [12].This results agreed with Lo cantor [13]. zhongyo [14]. Mimi Gduki [15]. Amani S [16]. Salvat A [17].

Here we describe the antimicrobial activity of *Trigonella foenum-graceum* extracts against different bacteria cause otitis media.We confirmed that the antibacterial activity of *Trigonella foenum - graceum* extract was dependant on selected bacterial extract, concentration extract, as adverse side effects have not been reported in any way of the clinical trials.

We apply the SAS statistical system to analyze the results that appear during the experiment with LSD (least significant

difference) value for bacteria (0.233) ,inhibition zone (0.309) and interaction (0.729), the results from table (1) shown that the *staphylococci* has more significant difference in their sensitivity for (Gentamicin, Tetracycline, Amikacin) than the other 3 test bacteria, because most of the genus *E. coli* shows some resistance to gentamycin and tetracycline [18]. Most of the strains of the genus *pseudomonas* were resistant to amino glycosides especially (Genta-mycin and Amikacin) and it also resists Tetracycline [19]. Most of the strains of the genus *Proteus* are resistant to gentamycin and highly susceptible to Amikacin [20]. and most of the genus *Proteus* resist tetracycline [21].

While the *Staphylococci* are susceptible for (gentamycin, amikacin, tetracycline) [22]. from the same table we saw that the Amikacin is the best antibiotic used among the other 6 antibiotics ,it gives the greater inhibition zones on the test bacteria and gentamycin and tetra-cyclin and then Rifampin ,and we realized that there is a great significant difference among all the test bacteria in association with inhibition zones formed by the Amikacin while in association with gentamicin there is no significant difference between *pseudomonas* and *proteus* but there is a significant difference between *E coli* and *staphylococci* and *pseudomonas proteus*, there is no significant difference between the bacterial inhibition zone formed by (Ampicilin, cephalothin, streptomycin) while in tetracyclin and rifampin there is no significant differences between the bacterial inhibition zones values just in S*taphylococci* in tetracyclin there is significant difference with the other test bacterial inhibition zones values and *E coli* in rifampin also there is significant difference with other test bacterial inhibition zones, this is because if the difference value between each two inhibition zones value for each two bacteria is more than (0.309) which is the LSD value for the inhibition zone, so it will be significant difference but if the difference is less than (0.309), so there is no significant difference.

We record the in hibition zones means, the greater mean is for the amikacin 19.25, 15.833 for gentamycin, 9.16 for tetracycline, 2.66 for rifampin. From the same table we record the interaction effect between the bacteria and the antibiotics , the *staphylococci* and the Amikacin has greater interaction value among the other test bacteria and antibiotics, Because the Amikacin show greater inhibition zones i.e. high antibacterial activity on all the tested bacteria ,while *staphylococci* Shawn high sensitivity to most of the test antibiotics (Gentamycin, tetracycline, Amikacin), so the difference between the inhibitions formed by the amikacin and the *staphylococcal* susceptibility Shawn significant difference more than (0.729) which the LSD value for interaction , the second interaction effect value between *E coli* and Gentamycin and then between *pseudomonas, protease* and tetracycline for the same reason .

The Bacterial inhibition zone mean are determined and the greater bacterial inhibition zone mean is for the *staphylococci* (9.619), because the *Staphylococci* shown greater inhibition zones values against most of the test antibiotics.

From figyre-5 which combine the sensitivity test by wells for both alcohol and water seeds extract with LSD value (0.05) , in the alcohol seeds extract the histogram shown the significant effect of the increasing the concentrations of the alcohol seeds extract for *trigonella foenum-graceum* on the inhibition of the bacterial growth , as a result of the increasing of the concentrations of the *trigonella foenum-graceum* from (200mg/ml to400mg/ml to 600mg/ml)

increasing of the concentrations of the *trigonella foenum- graceum* from (200 mg/ml to 400 mg/ml to 600mg/ml), there is a significant increasing in inhibition on the bacterial growth , the *E coli* has the greater inhibition zone value among all the test bacteria in the (600mg/ml) and also to the rest of the another concentrations because the greater concentration value contain concentrated herbal material that has highly antibacterial activity [23] . While in the water seeds extracts the histogram shown the same results with varying values less than the values obtained from the alcohol seeds extract but it indicates the same facts , as a result of increasing the concentration of the seeds extract (water, alcohol) it increase the inhibition of the microbial activity , and also *E coli* has more susceptibility or inhibition zone value among all the test bacteria after treating with the water seeds extract in concentration of (600mg/ml) and the rest of the another concentrations for the same reason of the alcohol seeds extract.

From fig(6) which combine the results of the MIC(minimum inhibitory concentration) for both the alcohol and water seeds extract of Trigonella foenum-graceum with LSD value (0.05), the MIC of the alcohol seeds extract shows variable activity on the test bacteria but it shows the same MIC value for both (*Staphylococci, Proteus*) and (*E.coli, pseudomonas*), but the highest MIC activity is occur on both E coli, *pseudomonas* because the Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints [24] ,while the MIC for the water seeds extracts of *Trigonella foenum-graceum* with LSD value is (0.05) , it shown variable MIC values but it's the same for (*Staphylococci, proteus, pseudomonas*) if we compare between the two extracts we find that they have the same MIC effect on the test bacteria but its differ in the *Pseudomonas*, the alcohol extract shown more activity on the *Pseudomonas* while the water seeds extract shown less MIC activity than the alcohol seeds extract.

The results of the present study support the usage of the studied plants and suggest that plant extracts possess compounds with antibacterial properties that can be further explored for antimicrobial activity. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious disease.

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