# The Effect Of Fenugreek Extract In The Growth Inhibition Of Some Bacteria *In Vitro*.

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الخلاصة

خلاص بذور نبات الحلبة بالكحول الاثيلي 70 % . وقد اختبرت تراكيز مختلفة منه في تتبيط نمويعض الجراثيم المرضية الموجّبة لصبغة كرام مثل(العنقودية الذهبية · Diplcoccus pneumonia ، المكورات الرئوية Staphylococcus aureus epidermidis البشروية Staphylococcusوالعقدية البرازية العنقودية والسالية (الاشيريكيه مثل کرام لصبغة (Streptococcus facials الزنجارية Klebsiella)والزائفة spp.)والكلبسيله(Escherichia القولونية(coli . ( ( Pseudomonas aeroginousa) حُيث أظهرت التراكيز العالية نسبيا (> 2 ملغم /مل) فاعليه في تثبيط نمو الجراثيم الموجبة لصبغة كرام ، وتميزت الجرائيم السالبة لصبغة كرام بكونها أقل حساسية في حين كانت الزائفة الزنجارية مقاومة له في جميع التراكيز المستخدمة . النتائج تؤكد أهمية مستخلص بذور نبات الحلبة في معالجة بعض الاخماج الجرثومية إضافة إلى أهميته في الصناعات الدوائية مما يشجع على تنقية المركبات الفعالة فيه واختبار فأعليتها في الزجاج ثم في الحي.

#### <u>Summary</u>

The seed of <u>trigonella Feneum</u> - <u>graecum</u> was extracted with 70% ethanol, the antibacterial activity of different concentration of the extract were tested to inhibition of some Gram positive pathogenicc bacteria such as <u>Staphylococcus aureus</u>. <u>Diplcoccus pneumonia</u>, <u>Staphylococcus epidermidis</u>, <u>Streptococcus facials</u> and Gram negative such as <u>Escherichia coli</u>, <u>Klebsiella spp</u> & <u>Pseudomonas aeroginousa</u>.

The high concentration  $\geq 2mg/ml$  showed antibacterial activity against gram-positive bacteria, while <u>pseudomonas</u> <u>auregenosa</u> was resistant to all concentration of extract.

The results indicated that the extract of fenugreek plant seeds is important in treatment of bacterial infection & drugs industries.

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#### **Introduction**

The medical plants were used for along time in treatment of many pathological cases, so that an increase in agriculture & uses of this plant for generations. The destination of scientific thinking to use this extracts properly that depending essentially on medical plants which contain biological active compounds to cure many different diseases, & has low side effects and price. The plant considers as vegetables protein source for human, animal and fish <sup>(1).</sup>

The Fenugreek with a scientific name Trigonella foenumgraceum belong to corneal family (Leguminoseae), one of these plant used as medical plants in drugs industries because it contain many soapy steroids as digitalis which is founded in seeds and used in preparation of different cortisones <sup>(2)</sup>. In addition it contains alkaloids such as trigonellin and scopoletine on nicotine and coumarine acid <sup>(3, 4)</sup>. The plant contains some amino acids, fats and mineral salts .It is used for treatment of loss of appetite and stimulation stomach.<sup>(5)</sup>

The Fenugreek plant acting on treatment of hyperglycemia and to lower high body temperature  $^{(6, 7)}$ , gases repellent, emollient and treatment of some intestinal helminthes  $^{(8)}$  in addition to effecting as relaxation substance and pain  $^{(9)}$ . The fenugreek plant used in treatment of some peptic ulcer cases  $^{(10)}$  and treatment of kidney stones in rats  $^{(11,12)}$ . It used in cure of bronchitis and asthma, seeds of plant causes increase in milk release in women during lactation  $^{(13)}$  and help in cure some wounds of diabetic patients ,intestinal infections ,diphtheria, tonsillitis  $^{(14)}$ . All that indicated to highly action of fenugreek plant as antibiotics.

The aims of this study is to present an alternative to the chemical antibiotics that safely acts and is economically cheep i.e. study of the effect of extract fenugreek seeds in inhibition of pathological bacterial in vitro.

#### **Materials and Methods**

The extract of ethanol alcohol 70% was prepared from fenugreek plant seeds by sahxulate methods <sup>(15)</sup> and makes different concentrations (0.25, 0.50, 1, 2, 4, 8, 16 mg/ml) by dissolving it in distilled water or sterilized the solution.

Use of Escherichia coli, Klebsiella Spp., Staphylococcus aureus, Diplococcus pneumonia, Staph. epidermidis, Streptococcus fecalis and Pseudomonas aeroginousa as experimental bacteria which be isolated and diagnosed  $^{(16)}$ .

Mueller – Hinton agar oxide glue employing in examination of sensitivity of experimental bacteria to different concentrations of ethanol alcohol fenugreek seeds extract when depending on Grove and Randall methods for this purpose.<sup>(17)</sup>

The experimental bacteria grow in broth agar for 24 h in  $37^{\circ}$  C & keep with darkness of McFarland solution no-(0, 5) and diluted 200 times to get final culture that contains (106-510) cell/ml than cultured on Mueller – Hinton agar oxide glue ,than put one drop from all concentration of ethylene alcohol extract of fenugreek seeds by using sterilized pipette through out metallic punch disc & transfer the disc on glue surface ( put one drop of distilled water in each punch disc for controlling) left for half an hour to dehydrate drops and remove disc, reput cover of container and incubate all containers for 24 h at  $37^{\circ}$  C. Three Petri dish was culture for each type of experimental bacteria kind.

The sensitivity of experimental bacteria determined by measuring of diameter inhibition zone for growth by putting drops from different concentration of ethylene alcohol fenugreek seeds extract.

The system which is used for this purpose is (Fisher – Lilly, antibiotic zone reader, Fisher scientific CompUSA).

### **Results**

 Table -1: Reveals the effect of Fenugreek plant seeds on the growth of pathological bacteria

Ethylene	Growth incubation rate (mg)						
Extract conc. (mg/ml)	E.Coli	Klebsiella Spp.	Staph. aureus	Diplo. pneumonia	Staph. epidermidis	Staph. Fecalis	Pseudomonas aeroginousa
0,25	-	-	-	-	-	-	-
0,5	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-
2	-	-	-	-	$0,6 \pm 8,6$	$0,2 \pm 8,5$	-
4	-	0,3±8,6	-	$0,3 \pm 8,8$	$0,4 \pm 9,3$	0,3 ±9,2	-
8	-	0,2±10,2	0,3±8,9	$0,2 \pm 10,2$	$0,2 \pm 11$	0,2 ±11,3	-
18	0,2 ±8,2	$0,1 \pm 11,5$	0,1±9,3	$0,3 \pm 12,6$	$0,2 \pm 13,9$	0,5 ±12,1	-

The 12% concentration of ethanol alcohol extract of fenugreek plant seeds is recognized by posted smell& white brown color, it acts as high concentration of antibiotic on bacteria, the concentration lower than 12% did not effect in growth of all experimental bacteria in this study. The first effect is inhibition of growth appearance in 2mg/ml concentration. The diameter of inhibition zone for staphylococcus epidermidis and streptococcus facials more than 8 ml for each type (table 1).

## **Discussion and conclusion**

The 70% concentration ethanol alcohol extract of fenugreek plant seeds considered as highly polarity ,therefore extract of active compounds almost such as alkaloids – steroid – soapy tribunal – flaphony effect on bacterial as antibiotic <sup>(18,19)</sup>. This may be due to resistance of Gram-ve bacteria or low sensitivity to this extract or related to uncompleted purification of this compounds or because the natural and acquired resistance of bacteria to this extract is related to inability to penetrate the fatty wall of bacteria.

These results conducted that the effect of 70% ethanol alcohol extract of fenugreek plant seeds act highly inhibition growth of gram positive & gram negative bacteria in vetro, these results conducted the importance of fenugreek plant seeds in treatment of infections such as tonsillitis, chest infections ,wounds & ulceration . In addition to importance in drugs industries because it contain active medical compounds. Purification and testing of action in vitro and in vivo. Our study fits together with <sup>(20)</sup> and assurance with <sup>(13)</sup> <sup>(14)</sup>. Fenugreek plant seeds extract used for treatment more than 26 types of pathological bacteria infections and considered one of broad spectrum antibiotics.

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