



In-vitro Comparative Study of Antibacterial activity of *Syzygium aromaticum* with three Antibiotics Against *E. coli*

Oday M. hady* Saad Saleem Raheem**
Ahmed Abbas hasan***

*Lecturer / Ph.D Medical Bacteriology, Department of Community Health, College of Health and Medical Technology, Kufa, Foundation of Technical Education

**Assist.lect. / Msc. Pharmacology and Toxicology, Department of Community Health, College of Health and Medical Technology, kufa, Foundation of Technical Education

***Assist.lect. / Msc. Medical Laboratories, Department of Pathological Analysis, College of Health and Medical Technology, kufa, Foundation of Technical Education

Abstract:

The results confirmed antibacterial activity with significant differences ($P \leq 0.05$) of clove (*Syzygium aromaticum*) in the concentration $10 \mu\text{g/ml}$ (MIC) (1.10 ± 0.31).

Also superiority of the antibiotic (Doxycycline) that has MIC (Minimum Inhibitory Concentration) at $10 \mu\text{g/ml}$ by significant differences ($p \leq 0.05$) (2.00 ± 0.08) against *E. coli* that appears sensitive to its, while it doesn't clear any significant differences in both of the antibiotics (Gentamicin and Gentadoxycycline). In case antibacterial activity of antibiotic (Gentamicin) against *E. coli* by sensitive tests observe resistance of *E. coli*, started from $10 \mu\text{g/ml}$ (1.60 ± 0.15) to $100 \mu\text{g/ml}$ (2.30 ± 0.05) by significant differences ($p \leq 0.05$). Also the antibiotic (Gentadoxycycline) by combination of (Gentamicin and Doxycycline) in-vitro, it doesn't show any significant differences ($p \leq 0.05$) when applied the sensitive tests of the antibiotic against *E. coli* at concentration $10 \mu\text{g/ml}$ (1.80 ± 0.15) and $100 \mu\text{g/ml}$ (2.60 ± 0.19), which demonstrates antagonist between them; by numbering of inhibition zone appear that the results less than (Doxycycline) and more than (Gentamicin), As a whole, these results illustrate superiority of Doxycycline as a antibacterial activity against *E. coli* depended on sensitivity tests according to antibiotics (Gentamicin and Gentadoxycycline) and clove (*Syzygium aromaticum*) by significant differences ($p \leq 0.05$).

Objective Knowing of antibacterial activity of clove (*Syzygium aromaticum*) against bacteria (*Escherichia coli*) in in-vitro, and compare of it with three antibiotics (Gentamicin, doxycycline and Gentadoxycycline), by measurement of inhibition zone for both of them.

Material and Methods Bacteria (*E. coli*) was isolated from the patients in the city of al-Sadir medical on E.M.B (Eosin Methylene Blue) agar and incubated at 37°C for 18-24 hours. This trial is performed in in-vitro (sensitivity test) by agar well diffusion technique, in diameter of well (8 mm) and by measurement of inhibition zone in nutrient agar after 24 hrs, and knowing of Minimum Inhibitory Concentration (MIC) for both of them.

Conclusions *Syzygium aromaticum* has high antibacterial activity against the Bacterial growth of (*Escherichia coli*). Doxycycline has high antibacterial activity against the Bacterial growth of (*Escherichia coli*). Resistance of *E. coli* to the commonly used eradication antibiotic drug (Gentamicin). The combination between two antibiotics (Gentamicin and doxycycline) doesn't give antibacterial activity against the Bacterial growth of (*Escherichia coli*).

Keyword: (Clove), *Syzygium aromaticum*, Antibiotic, Gentamicin, Doxycycline, Gentadoxycycline

دراسة مقارنة مختبرية للفعالية المضادة للبكتريا للقرنفل مع ثلاثة انواع من المضادات الحيوية ضد بكتريا الاشيريكييا كولاي

عدي متعب هادي* سعد سليم رحيم**
أحمد عباس حسن***

* مدرس/ دكتوراه أحياء مجهرية طبية، قسم صحة مجتمع، كلية التقنيات الصحية والطبية، الكوفة، هيئة التعليم التقني
** مدرس مساعد/ ماجستير أدوية وسموم، قسم صحة مجتمع، كلية التقنيات الصحية والطبية، الكوفة، هيئة التعليم التقني
*** مدرس مساعد/ ماجستير مختبرات طبية، قسم التحليلات المرضية، كلية التقنيات الصحية والطبية، الكوفة، هيئة التعليم التقني

الخلاصة:

أكدت النتائج بأن للقرنفل (*Syzygium aromaticum*) فعالية ضد بكتريا الـ (*E. coli*) وبفروقات معنوية ($p \leq 0.05$). وبينت النتائج تفوق المضاد الحيوي (Doxycycline) بالمقارنة مع بقية المضادات الحيوية (Gentamicin و Gentadoxycycline) والقرنفل (*Syzygium aromaticum*) بتركيز (10 µg/ml) MIC (2.30 ± 0.05). لم تظهر أي فروقات معنوية ($p \leq 0.05$) عند خلط مضادين حياتيين في المختبر (Doxycycline و Gentamicin) من خلال قراءة منطقة التثبيط وعند تركيز (10 µg/ml) (1.80 ± 0.15) وهي اقل من الـ (Doxycycline) وأكثر من الـ (Gentamicin).

الهدف: معرفة الفعل المضاد البكتيري للقرنفل (*Syzygium aromaticum*) ضد بكتريا الـ (*Escherichia coli*)، ومقارنتها مع ثلاثة انواع من المضادات الحيوية (Gentamicin, doxycycline and Gentadoxycycline)، من خلال قياس منطقة التثبيط لكل واحد منهم مختبريا.

طريقة العمل: عزلت بكتريا الـ (*E. coli*) من مدينة الصدر الطبية على وسط انتخابي agar (Eosin (E.M.B) Methylene Blue) agar ووضعت في الحاضنة عند درجة 37 م⁰ للفترة 24 ساعة، وبعد ذلك أجريت لها فحوصات حساسية لكل من (*Syzygium aromaticum*)، Gentamicin, doxycycline and Gentadoxycycline بتقنية انتشار في الحفر في وسط هلام الاغار، وقطر الحفرة (8 mm)، ومعرفة اوطأ تركيز مثبط لنمو البكتريا من خلال قياس منطقة التثبيط لكل منهم في الوسط المغذي بعد 24 ساعة من الحضانة.

Introduction:

Escherichia coli O157:H7 is a concern to public health on a global scale [1] and is found in a wide variety of foodstuffs including meat and meat products, milk, yogurt, water, salad vegetables, fruits, fruit juices and cider [2;1]. Pasteurization and cooking are adequate methods of ensuring that viable cells are eliminated, but heat treatment is not desirable for all foods and cross-contamination cannot always be prevented. Controlling the numbers and growth of *E. coli* O157:H7 therefore remains an important objective for sectors of the food production industry. Essential oil of clove, dispersed (0.4%v/v) in a concentrated sugar solution, had a marked germicidal effect against various bacteria and *Candida albicans*. *Staphylococcus*

aureus (five strains), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, and *Escherichia coli* inoculated at a level of 10^7 cfu/ml, and *C. albicans* (inoculum 4.0×10^5 cfu/ml) were killed (< 99.999%) after 2–7 min in a laboratory broth supplemented with 63% (v/w) of sugar, and containing 0.4% (v/w) of essential oil of clove. Added organic matter (i.e. human or bovine serum) did not impair its antimicrobial activity [3]. Now microorganisms have become resistance to many antibiotics due to the misusing of drugs, which is decreasing efficiency of conventional medicines[4]. The development of antibiotic resistance in bacteria is of public concern in view of the fact that a patient could develop antibiotic

resistance by contacting a resistant microorganism or the emergence of a microorganism in the patient's body when treatment with antibiotic begins [5]. Chemical constituents with antioxidant activity found in high concentrations in plants determine their considerable role in the prevention of various degenerative diseases [6;7]. *Syzygium* species (Fam. Myrtaceae) have been reported to possess antibacterial [8]. and anti-inflammatory activity [9]. It was reported that the buds of *Syzygium aromaticum* (L.) Merr. & Perry (clove) were used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity [10]. The antimicrobial activity of the essential oils from clove and rosemary (*Rosmarinus officinalis* L.) has been tested alone and in combination [10].

In addition, the antimicrobial activity of clove essentials oil have been studied against a large number of multi-resistant *Staphylococcus epidermidis* as well as the composition of the oil by GC/MS analysis [11]. Cloves are used as carminative, to increase hydrochloric acid in the stomach and to improve peristalsis[12].

Objective

Knowing of antibacterial activity of clove (*Syzygium aromaticum*) against bacteria (*Escherichia coli*) in in-vitro, and compare of it with three antibiotics (Gentamicin, doxycycline and Gentadoxycycline), by measurement of inhibition zone.

Material and Method:

In- vitro study

Equipments

(Table 1): General Laboratory Equipments

List	Instruments	Company	Origin
1	Autoclave	JSR	Korea
2	Water path	JSR	Korea
3	Incubater	Memmert	Germany
4	Electric oven	Memmert	Germany
5	Light microscope	Olympus	Japan
6	Sensitive balance	Adam	
7	Hood	JSR	Korea
8	Refrigerator	LG	Korea
9	Glassware	-----	-----
10	Freezer	LG	Korea
11	Microprocessor PH meter	HANA 211	Romania
12	Hand held refractometer	National Industrial Supply	USA
13	micropipette	Gilson	Frans
14	Slides	Meheco	China
15	Sterile syringes 1,5,15	Laleh	Iran
List	Instruments	Company	Origin
16	Insulin syringe 1 ml	-----	China
17	Petri. dishes	Iraq-local market	Iraq
19	Loop	Himedia	India
20	Plastic rack	Meheco	China
21	Gas burner	-----	China
22	Roller mixer	Jemy industrial corp	Taiwan

Culture Media:**Brain Heart Infusion agar**

It consists of Calf Brains, Infusion , Beef Heart, Infusion , Proteose Peptone , Dextrose , Sodium Chloride and Disodium Phosphate. This medium is a highly [nutritious](#) general-purpose [growth and storage medium](#). It was prepared by manufacture company *Himedia* by Suspend 52 g of the medium in one liter of purified water and heat with frequent agitation and boil for one minute to completely dissolve the medium and Autoclave at 121 °C for 15 minutes.

Nutrient agar

It consists of beef extract, yeast extract, [peptone](#) , and [agar](#). Nutrient agar is a microbiological [growth medium](#) commonly used for the routine cultivation of [non-fastidious](#) bacteria. It is useful because it remains solid even at relatively high temperatures. Also, bacteria grown in nutrient agar grows on the surface, and is clearly visible as small colonies. prepare by manufacture company *Himedia* by Suspend 28 g of the medium in one liter of purified water and Autoclave at 121°C for 15 minutes.

Eosin Methylene Blue

Suspend 35.96 gms in 1000ml distilled water, heat to boiling to dissolve the medium completely, sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes, avoid overheating, cool to 45-50 °C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the Inoculent precipitate (if ENB agar is inoculated on the same day, it may be used without autoclave sterilization).

Antibacterial agents

- 1 . Gentamicin, ampoule 80 mg /2 ml , Meheco company, china.
2. Doxycycline, yellow powder, capsule 500 mg, Samarra company , Iraq.
3. Gentadocycline, prepared in-vitro as 100mg / ml, by combination of two antibiotics (Gentamicin and Doxycycline).

Methods**Identification of bacteria*****E. coli* :**

The bacteria *E coli* is harvested from the patients in the city of al- Sadir medical. The organism was subcultured on E.M.B (Eosin Methylene Blue) agar and incubated at 37 °C for 18-24 hours (picture 1).

Sensitivity Tests

Measurement of *Syzygium aromaticum* antimicrobial activity with different concentration by the use of agar well diffusion techniques against *E. coli*, and compared with three antibiotics – Gentamicin , Doxycycline and Gentadocycline with different concentration[13].

Preparation the *Syzygium aromaticum* :

The *Syzygium aromaticum* prepared as following:

- Clove (*Syzygium aromaticum*) is prepared as essential oil and water extraction technique [16].
- The stock solutions of a *Syzygium aromaticum* were prepared by dissolving 10 gm of its in 100 ml of hot distal water (w/v).

Preparation the antibiotics:

1. Gentamicin 80 mg /2ml : taken the 0.8 ml of antibiotics and then completed to 10 ml distal water in order to getting (100 µg /ml) (stock solution) ,and from it premedicated the different concentration : 10,20, 40 ,60,and 80 µg /ml .
2. Doxycycline 100 mg capsule: dissolving the capsule in 10 ml of distal water and then taken 0.1 ml of antibiotics and completed to 10 ml distal water in order to getting the (100µg / ml) and from it premedicated the different concentrations as follow : 10, 20, 40, 60, and 80 µg /ml.
4. Gentadocycline : prepared in-vitro by combination of Gentamycin and Doxycycline as tock solution (100 µg / ml), and from it premedicated the different concentrations as follow : 10, 20, 40, 60, and 80 µg /ml.

Bacterial count:

E coli is counted by:

- McFarland Solution (tube No .0.5 (Standard McFarland solution No 0.5.was prepared according to [14] as follows:

- Solution (A) this was prepared by dissolving 1.175 gm of barium chloride ($BaCl_2 \cdot 2H_2O$) in 100 ml distilled water.
- Solution (B) this was prepared by adding 1 ml of concentrated sulfuric acid (H_2SO_4) in 100 ml distilled water. The two solutions were mixed by the addition of 0.5 ml from solution A to 99.5 ml from solution B. The prepared solution was used to compare the turbidity of bacterial suspension to obtain an approximate cell density of 1.5×10^8 cell / ml.

Bacterial inoculation of media

Nutrient agar has been prepared after sterilized with autoclave 15 minutes at $121^\circ C$ and 15 IbS, nutrient agar by swap that contain colonies of (*E . coli*) from macFarland solution (tube No. 0.5) that contain 1.5×10^8 cell / ml., then followed:

Sensitivity of *Syzygium aromaticum* and antibiotics to the experimental bacteria (*E . coli*) performed by Wells of 8 mm in diameter were then made in the solid medium with a metallic device and filled with the different concentrations of *Syzygium aromaticum* of each sample (100 μL / well). The plates containing bacteria were incubated at $37^\circ C$ for 24 h. After incubation, the diameter of inhibitory zones was measured in mm [13].

Statistical Analysis

All the statistical analysis have been performed by the SPSS 8.0 statistical package. The values between groups have been compared by independent sample -f-test and one- way ANOVA (analysis of variance). P values less than or equal to 0.05 and 0.01 have been evaluated (Least Significant Difference LSD) as statistical significant [15].

Results:

The results in table (2), the results show antibacterial activity with significant

differences ($P \leq 0.05$) of clove (*Syzygium aromaticum*) in the concentration $10 \mu g/ml$ (1.10 ± 0.31). [picture 2,3,4][figure 1,2,3,4].

In table (3), show superiority of the antibiotic (Doxycycline) that has MIC at $10 \mu g/ml$ by significant differences ($p \leq 0.05$) (2.00 ± 0.08) against *E . coli* that appears sensitive to its, while it doesn't clear any significant differences in both of the antibiotics (Gentamicin and Gentadoxycycline). In case antibacterial activity of antibiotic (Gentamicin) against *E . coli* by sensitive tests observe resistance of *E . coli*, started from $10 \mu g/ml$ (1.60 ± 0.15) to $100 \mu g/ml$ (2.30 ± 0.05) by significant differences ($p \leq 0.05$). Also the antibiotic (Gentadoxycycline) by combination of (Gentamicin and Doxycycline) in-vitro, it doesn't show any significant differences ($p \leq 0.05$) when applied the sensitive tests of the antibiotic against *E . coli* at concentration $10 \mu g/ml$ (1.80 ± 0.15) and $100 \mu g/ml$ (2.60 ± 0.19), which demonstrates there is no existence any synergism between them; by numbering of inhibition zone appeared that the results less than (Doxycycline) and more than (Gentamicin), As a whole, these results illustrate superiority of doxycycline as a antibacterial activity against *E . coli* depended on sensitivity tests according to antibiotics (Gentamicin and Gentadoxycycline) and clove (*Syzygium aromaticum*) by significant differences ($p \leq 0.05$) [picture 2,3,4] [figure 1,2,3,4].

Discussion:

There are different significant ($p \leq 0.05$) of antibacterial activity of clove (*Syzygium aromaticum*), and disagreement with previous study, it is certain [16] that the active concentration of this clove against *E . coli* is ($400 \mu g /ml$), due to antiinflammatory, and antioxidant [11]. This study shown resistance of Gentamicin by *E . coli* due to several pathways of resistance which is can be caused by

decreased uptake of drug when the oxygen-dependent transport system for aminoglycosides or porin channels are absent, and plasmid-associated synthesis of enzymes (for example, acetyl transferases, nucleotidyltransferases, and phosphotransferases) that modify and inactivate aminoglycoside antibiotics. Each of these enzymes has its own aminoglycoside specificity; therefore, cross-resistance is not an invariable rule[17]. Antibiotic (Doxycycline) has high antibacterial activity against *E . coli* by numbering of inhibition zone among them (Gentamicin, Gentadocycline and *Syzygium aromaticum*), due to this drug binds reversibly to the 30S subunit of the bacterial ribosome, thereby blocking

access of the amino acyl-tRNA to the mRNA-ribosome complex at the acceptor site, and as broad-spectrum, bacteriostatic antibiotics, the tetracyclines are effective against gram-positive and gram-negative bacteria as well as against organisms other than bacteria[17].gentadocycline gives few active against bacteria (*E . coli*), where it is less than doxycycline and more than Gentamicin by the measurement of inhibition zone through agar well diffusion technique, because of Gentamicin is bacteriocidal effect of antibacterial while Doxycycline is bacteriostatic effect, both of them protein synthesis inhibitors and bind to the 30S subunit of the bacterial ribosome[18].



Picture (1),show culture of *E coli* on Eosin Methylene Blue (E.M.B) .

Concentration	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	60 mg/ml	70 mg/ml	80 mg/ml	90mg/ml	100mg/ml
<i>Syzygium aromaticum</i>	1.10±0.31 A	1.50±0.05 B	1.70±0.05 C	1.90±0.09 D	2.10±0.05 E	2.10±0.12 E	2.20±0.17 F	2.30±0.18 G	2.40±0.22 H	2.50±0.22 I

Table (2). Diameter zone of inhibition (mm.) of different types of *Syzygium aromaticum* against *E. coli*

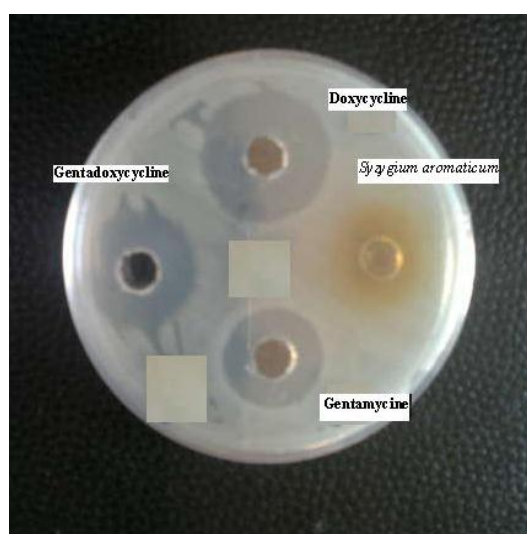
-Different capital refer to significant differences between groups horizontally (P≤0.05)
-The values represent Mean ± SE

Concentration Antibiotics	10 µg /ml	20 µg /ml	30 µg /ml	40 µg /ml	50 µg /ml	60 µg /ml	70µg /ml	80 µg /ml	90 µg /ml	100 µg /ml
Gentamycin	1.60±0.15 Aa	1.60±0.13 Aa	1.90±0.05 Ca	1.90±0.08 Ca	2.00±0.05 Da	2.00±0.04 Da	2.20±0.08 Ea	2.20±0.09 Ea	2.20±0.05 Ea	2.30±0.05 Fa
Doxycycline	2.00±0.08 Ac	2.20±0.08 Bc	2.50±0.05 Cc	2.50±0.07 Cc	2.60±0.04 Dc	2.70±0.05 Ec	2.80±0.05 Fc	2.90±0.09 Gc	2.90±0.09 Gc	3.00±0.11 Hc
Gentadoxycycline	1.80± 0.15 Ab	1.80±0.18 Ab	2.30±0.05 Bb	2.30±0.05 Bb	2.40±0.05 Cb	2.50±0.09 Db	2.50±0.04 Db	2.50±0.05 Db	2.60±0.05 Eb	2.60±0.19 Eb

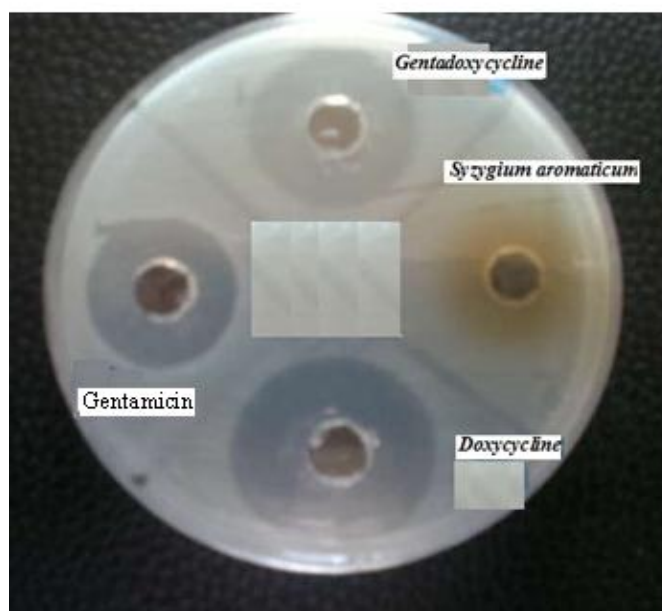
Table (3). Diameter zone of inhibition (mm.) of different antibacterial agents at different concentration against *E coli*.

-Different capital refer to significant differences between groups and within groups horizontally ($P \leq 0.05$).

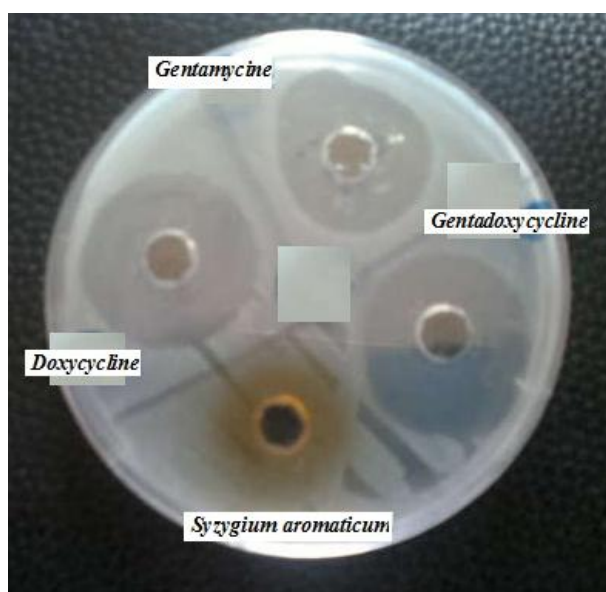
-The values represent Mean \pm SE



Picture(2), show sensitivity tests of Antibiotics (Gentamicin, Doxycycline, and Gentadoxycycline at 70 µg /ml, and *Syzygium aromaticum* at (30 mg/ml)



Picture(3),show sensitivity tests of Antibiotics (Gentamicin, doxycycline,gentadoxycycline and Gentadoxycycline at **80 µg/ml**), and *Syzygium aromaticum* at (**80 mg/ml**).



Picture(4),show sensitivity tests of Antibiotics (Gentamicin ,doxycycline,gentadoxycycline and Gentadoxycycline at **100 µg/ml**),and *Syzygium aromaticum* at (**100 mg/ml**).

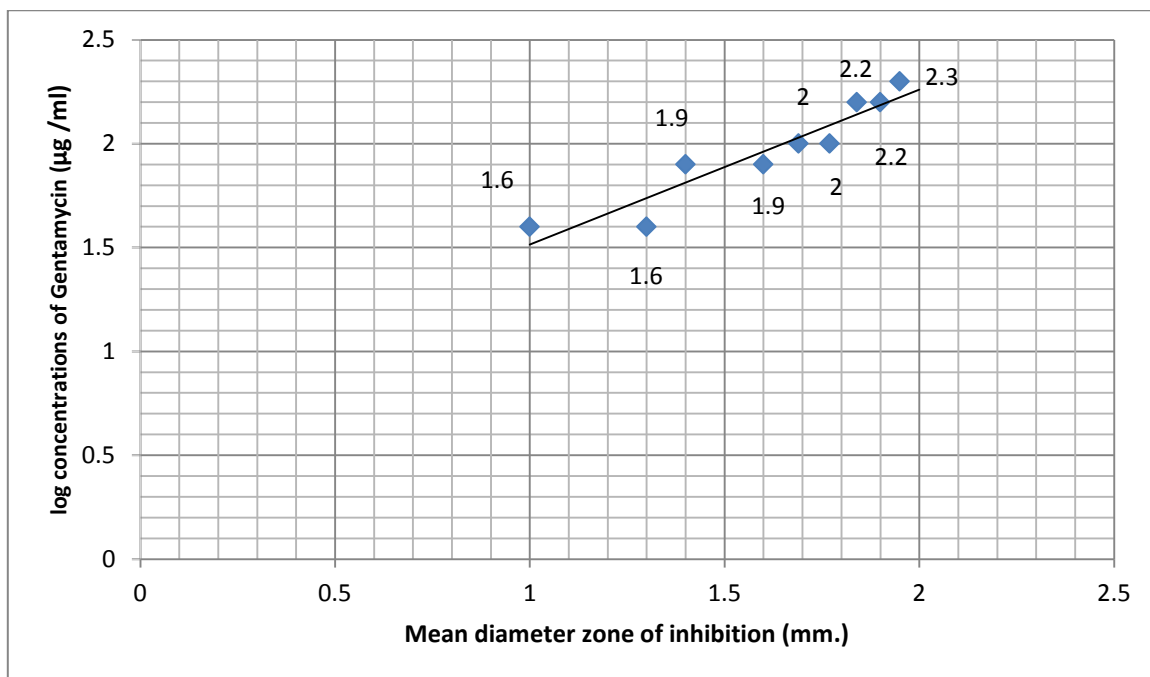


Fig. (1). Proportional relationship between log concentrations of Gentamycin and mean diameter zone of inhibition (mm.) against *E coli*

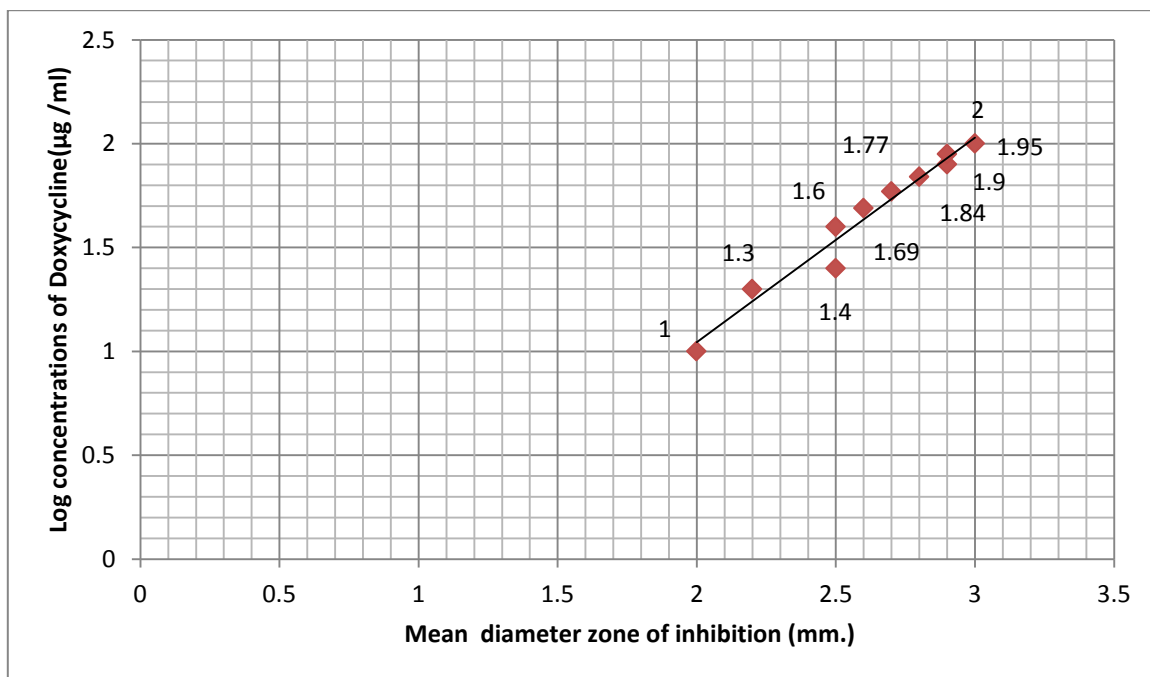


Fig. (2). Proportional relationship between log concentrations of Doxycycline and mean diameter zone of inhibition (mm.) against *E coli*

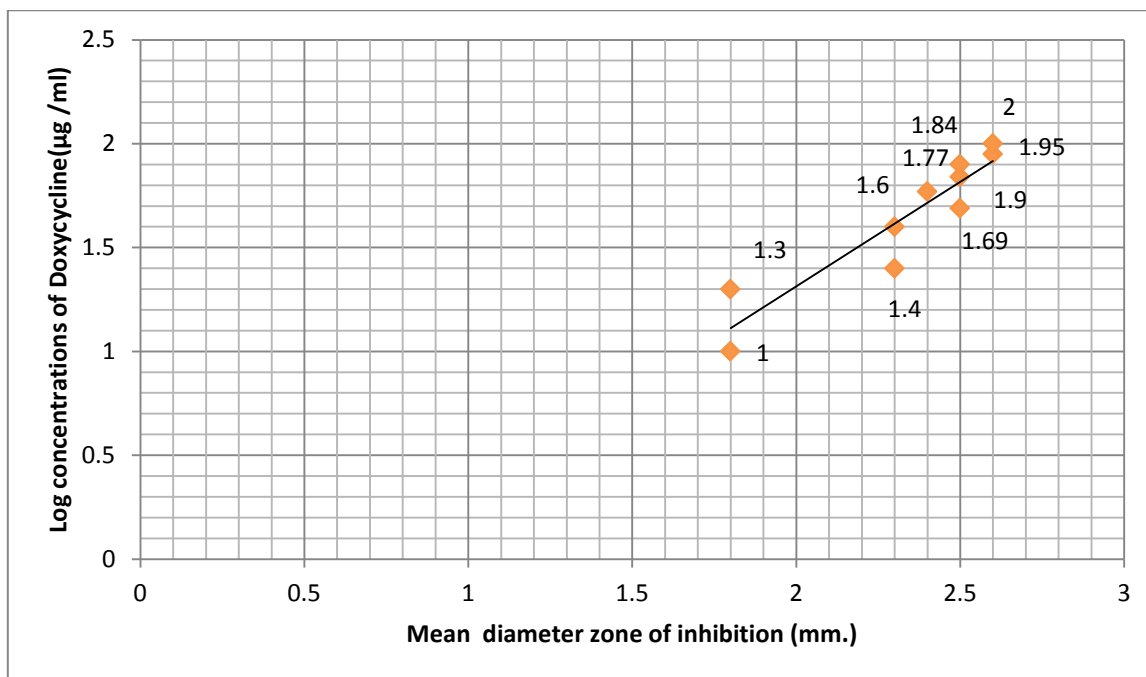


Fig. (3). Proportional relationship between log concentrations of Gentadoxycycline and mean diameter zone of inhibition (mm.) against *E coli*

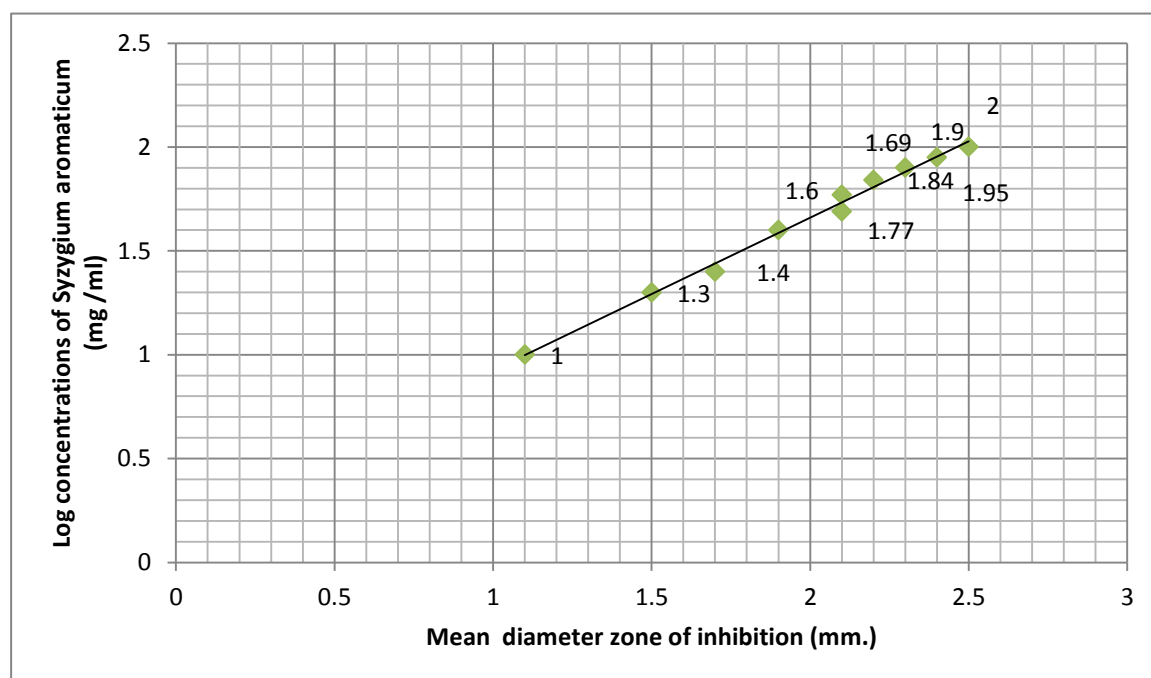


Fig. (4). Proportional relationship between log concentrations of *Syzygium aromaticum* and mean diameter zone of inhibition (mm.) against *E coli*

Conclusions:

- It can be included from the present study, that *Syzygium aromaticum* has high antibacterial activity against the Bacterial growth of (*Escherichia coli*).
- Doxycycline has high antibacterial activity against the Bacterial growth of (*Escherichia coli*), by numbering of inhibition zone .
- Resistance of *E.coli* to the commonly used eradication antibiotic drug(Gentamicin), which due to wide spread use of antibiotics for infection and different pathways resistance of bacteria towards its .
- The combination between two antibiotics (Gentamicin and doxycycline) doesn't give antibacterial activity against the Bacterial growth of (*Escherichia coli*).

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