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EFFECT OF SOME ANTIBIOTICS AND *TRIGONELLA FOENUM* L. PLANT EXTRACT ON BACTERIA ISOLATED FROM PREGNANT WOMEN

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Abstract

A total (126) sample included (76) samples administered from pregnant women and 50 samples from non-pregnant women whose ages range from (15-45) years ,results showed the samples that gave bacterial growth were (68) samples, at a rate of 54%, while they did not give (58) samples and by 46%, Positive isolates were diagnosed from pregnant women and are *Staph. epidermidis, Staph. aureus*, *Staph. saprophyticus.* As for non-pregnant women, only isolation *Staph. epidermdis*. The difference in the age groups response to bacterial vaginosis, where the highest percentage was within the age limits 25-35 years, with a rate of 47%, also results showed that *E. coli* isolates showed resistance to all antibiotics except for CIP ,and a clear difference between bacterial isolates in their resistance and sensitivity to the aqueous and alcoholic extract of *Trigonella foenum and Origanum marjorana* plants, as sensitivity differed with the different concentrations used from the extracts, as the highest values of the diameter of the accretions at the concentration were 100% for both plants.
***Keywords*:** *Trigonella foenum,* plant extract, antibiotics bacteria.

Introduction

Bacterial Vaginitis (BV) is one of the female genital diseases, the most common in women during pregnancy and childbirth (Virginia *et al.,* 2000) and it is also part of the female reproductive system (RTI) Reproduction tract infection. (Muckinng 2007). There are many causes of vaginitis, including bacteria. The condition is called bacterial vaginosis, which is an imbalance as well as between the anaerobic bacteria that cause inflammation (Martirosion, 2004).

Bacterial vaginosis causes a lot of real problems in the pregnant mother, which leads to serious complications, including abortion, premature births, amniotic fluid surrounding the fetus, early rupture of the fetal membrane, and low fetal weight in childbirth (Girod *et al.*, 2012). These causes all cause high death rates before Childbirth (Briry *et al.*, 2011).

The resistance of pathogens to antibiotics has increased over time, and the excessive and continuous indiscriminate use of antibiotics leads to the production of bacterial strains resistant to antibiotics and the spread of the trait of resistance (Ali, 2010; Kenann & Chou, 2017), which called for the need to pay attention to scientific research to find safer and more effective alternatives resisting sick neighborhoods (Junaid *et al*., 2016). Hence the idea of returning to medicinal plants which have a significant and important role in human life, due to the multiplicity, and types of its wide range of uses (Al-Hadwani, 2004; Abdel-Hussein, 2016) as these plant extracts contain many compounds, including organic acids, aromatic compounds, coumarins, flavonoids, tannins, alkaloids, glycosides, terpenoids, steroids, in addition to most toxic gases (Purtnam, 1987). There are many plants that have been studied, such as plants, that are pharmacologically effective for containing them effective compounds have a clear biological effect against bacteria and theories various (Oribi, 2017), including *Tigonella foenum* and *Origanum marjorana*

Materials and Methods

A total (126) samples were taken ((76) from pregnant women and (50) samples from non-pregnant women)whose ages range from (15-45) years from the recumbents in Salah Al-Din General Hospital-Tikrit Governorate/Iraq, the samples were collected by sterile Cotton swab. They are ready and quietly enter the vagina and will be moved in a circular motion and gently for 10-5 seconds to absorb pus and other vaginal fluids. The samples were planted on the blood media, the salt mannitol medium, and the macaque medium. The dishes were incubated at a temperature of 37 °C for 48-24 hours, after which the isolates were purified and incubated antenna at a temperature of 37 °C for a period of 24 hours ,the initial diagnosis of the isolated bacteria was carried out depending on the colony's color and shape, its size, the type of decomposition it brought about and the edge and whether it was fermented or non-fermented for lactose sugar, and the pure isolates were preserved on the center of the 4-sloping acids for conducting phenotypic and biochemical tests for them, then cultural media prepared depending to the information recorded on the package by supplied company then sterilized by autoclave on 121 ºC for 15 minutes and pressure 15 pound. The media added to sterilized dishes and put in the incubator at 37 ^{0}C for 24 hours then kept in the refrigerator until uses. the media which prepared were Muller Hinton Agar medium, Nutrient Broth media, Manitol salt Agar medium, MacConkey Agar and Brain heart infusion broth while prepared reagents and solutions according (Collee *et al.*, 1996)were Gram stain solutions, Normal salin solution and Mcfarland standard solution.

Identification Bacterial : The slice examination of the isolates under study was carried out by taking a smear using the bacterial loop from the colonies of each bacterial farm and dyeing with a dye-stained color.

Antibiotics Sensitivity Test: Isolated bacteria sensevity tested for (7) antibiotics Amoxicilin (AX), Ampicilin (AM), Aztronam (ATM), Ciprofloxacin (CIP), Cifixime (CFM),

Erthromycin and Gentamycin (GN) according to standard Kirby-Bauer method (Lepp, 2010).

***Preparation of plant Extracts:** Two types of solvents were used in the extracts from plants (aqueous, alcoholic) and crushed the seeds and leaves of each plant, after which the extracts were prepared from them.

Plant Extract Preparation: Aqueous extract prepared by mixing (40) gm from plant powder with (60) cm^3 distill water according to (Riose *et al*., 1987), while Ethanolic extract prepared by mixing (20) gm from plant powder with (200) cm^3 of Ethylin alcohol (90%)according to (Grand *et al.*, 1988).

Plant Extract Inhibition Effective Test: The diffusion method was used in the agar diffusion method by drilling wells to test the bacterial sensitivity of plant extracts at standard concentrations (100, 50, 25 mg / mL in the pit from the food medium) (Egorore, 1985). The method involved making three pits of equal dimensions in the Muller / Hinton steel medium Diameter 6mm with a Cork borer to accommodate plant solutions with a value of (0.2) ml for each stage after implantation (0.1) ml of bacterial strand on the medium, the dishes were left in the refrigerator for one hour for the purpose of spreading the solutions of plants, then incubated the dishes in the incubator at a degree of 37 for a period of 24 hours and the results were read by measuring the diameter of the inhibition zone (Inhibition zone) (Sexena *et al*., 1999)

Results and Discussion

The results in table (1) appeared there were (62) sample by (81.5%) gave bacterial growth and (14) sample by (18.5%) did not give any growth of pregnant women, while only (6) sample by (12%) gave growth and (44) sample by (88%) did not give growth of non pregnant women. The results in agreement with Al-Obaidi (2007) and Al-Obaidi (2017), The difference in percentage of bacteria growth between pregnant and non pregnant women due to physiological factors connected with pregnant which consider suitable and favorite for bacteria growth, also it connected with non drug taking by pregnant women for fear on embryo during pregnant period ,or the microorganism may be virus or fungi or non aerobic organism which its growth need special cultural media or to nature of taken samples and its size or difference in temperature or humidity (Brain-Garcia & Whitmore, 2008; Al-Obaidi, 2017), another causes as taking antibiotics and anti-pregnant tablets which encourage bacteria infection process and appearance pathogenic symptoms as vaginal secretions, itch and change in acidity number (pH) in addition to smell (Gutmen *et al.*, 2005), and disorder in Estrogen hormone in non-pregnant women which act to convert the Glycogen to lactic acid which make the (pH) acidity for vaginal (Ali, 2011). Identification of negative bacteria for gram stain appeared in pregnant women showed *Staph. epidermidis, Staph. aureus*, *Staph. saprophyticus* bacteria by 35.5, 32.3, 6.5% respectively, and only *Staph. epidermidis* in the non pregnant women by 33.4% (table 2),while positive bacteria in pregnant women were *E. coli, P. aeruginosa, P. flourescens, K. pneumonia, Aero. salmonicidia, Aero. caviae and Prov. stuartii* by (12.9, 3.2, 3.2, 1.6, 1.6, 1.6, 1.6, 1.6%) and only *E. coli* in the non pregnant women by (66.7%) (table 3). The results is near to Al-Obaidy (2017) and agreement with Al-Ghanam (2018) whom isolated and identified same bacteria from (UTIs).

Table 1 : First isolation percentage of urea in pregnant and non pregnant women.

Culture results	Samples		Pregnant		Non pregnant	
	Total	(%)	No.	(%)	No.	(%)
Growth	68	54	62	81.5	6	12
Non Growth	58	46	14	18.5	44	88
Total	126	100	76	100	50	100

Table 2 : Negative bacterial isolations percentage in pregnant and non pregnant women.

Pregnant			Non Pregnant		
Isolates	No.	(%)	Isolates	No.	(%)
S. epidermidis	22		*S. epidermidis*	2	33.4
S. aureus	20				
S. saprophytic	4				

Table 3 : Positive bacterial isolations percentage in pregnant and non pregnant women.

Pregnan			Non Pregnant		
Isolates	No.	(%)	Isolates	No.	(%)
E. coli	12.9		*E.coli*	4	66.7
P. aeruginosa	3.2				
P. flourescens	3.2				
K. pneumomia	1.6				
Aero . salmonicidia	1.6				
Aero. caviae	1.6				
Prov. stuartii	1.6				
Acin. lowffii	1.6				

Table (4) shows the difference in the response of age groups to bacterial vaginitis, where the highest percentage was within the age limits of 25-35 years, with a rate of 47%, while the lowest percentage of bacterial vaginosis was in the age group 35-45 and reached 23.5%, and these results it meets Al-Samurai (2009) and Al-Obaidi (2017), where their

highest percentage was in the age group (25-35) years that was the percentage has 49.79% and 48.38%, while our results differed with Jaber (2008), which shows that the highest rate of infection was at the age group (44-53) years, at 82.1%. Acikgoz *et al.* (2002) indicated that age is one of the factors responsible for the natural variation of the (flora) of the vagina and is the cause of the periodic appearance of some pathogenic microbes. It also secretes a high incidence in the age group (25-35) Genetic hormones reach high levels during this period, and the increase and decrease in the amount of estrogen from the normal level leads to weakening of the contraction of the bladder muscle and ureters, which leads to the return of damage and the occurrence of infections (Al-Ani, 2005, Al-Obaidi, 2017), and the reason may be attributed to the number of samples taken, the table has revealed the occurrence of injuries, but to a lesser extent in the older age groups (34-45) The reason for the occurrence of infection in this elderly age group is due to the weak resistance of the body and its regression in the general health level in addition to a decrease in the hormone progesterone in elderly women, causing weakening of the muscles of the bladder and ureters. Which, in turn, increases the probability of a return of urinary tract flow and backwards flow through the ureter to quantity (Schmidit & Robert, 2005, Al-Obaidy, 2007).

Table 4 : Percentage of vaginal isolates bacteria according age category

Age category	Number	Percentage
15-25	20	29.9
25-35	20	47.0
35-45	16	23.5

The results on table (5) showed that the isolates differ in their sensitivity and resistance to antibiotics depending on the method of spreading from the disk and measuring the diameter of the area of dulling around the antibiotic tablets used to determine the sensitivity of the bacterial isolates under study to the antibiotics. The results of our study showed that *Staph. aureus* resistant for the antibiotic (AX), (AM) and (ATM), while sensitive to the remaining antibiotics and the most sensitive to antibiotics (CIP) was with a nominal diameter of (30) mm. Table, and these results are consistent with Al-Obaidi (2017), who indicated resistance isolation for antibiotic (AM) as agreed with Ali (2011) who demonstrated the sensitivity of the *Staph. aureus* for antibiotics (GN), (AM), and (AX) and agreed with Al-Jubouri (2012) which indicated isolation resistance to the antibiotic (AM) . *Staph. epidermidis* is resistant to most antibiotics used except antibiotic sensitivity (CIP), (GN),and *Staph. Saprophytic* isolation resistance to antibiotics used between antigen and photosensitive, as antibiotic resistance (AX), (ATM), (CFM), (E) and It was more sensitive to CIP with inhibition diameter reached (32) mm. These results do not agree with it with Al-Obaidy (2017) respect to the antibody (AM), nor do they agree with Al-Dawoodi (2018), who indicated isolation resistance to the antibiotic (AM). The results of the same table indicate that *E. coli* isolates showed resistance to all antibiotics used except CIPs with a diameter of (30) mm. The results were in agreement with Jarjis (2006), which shows that isolates showed absolute resistance to antibiotics used, as agreed with Al-Obaidi (2017), which indicated resistance to isolation *E. coli* for antibiotics (AM).

Table 5 : Inhibition diameter of isolates treated with some antibiotics

Antibiotics / Isolates	AX	AM	ATM	CIP	CFM	E	GN
Staph. aureas	0	17	0	30	0	15	20
Staph. epidermidis	0	0	0	19	0	11	20
Staph. saprophytic	0	20	0	32	13	0	21
E. coli	0	0	0	30	0	0	20
P. aeruginosa	0	32	11	30	0	0	20

Results in table (6) refer to clear difference between isolated bacteria on its resistant to aqueous and alcoholic extract of *T. foenum* and *O. majorana* plants. The highest value of inhibition zone were at 100% concentration for both extracts, and there were difference among isolated bacteria on its response to the extracts, the high value of inhibition zone reached (33) and (35) mm for the *S. aureus* bacteria while the lest value were (23) and (24)mm for *E.coli* on both extracts. The variance among bacteria isolated due to difference among the species and the kind of active ingredients on the plant extracts (Mitscher *et al.*, 1972), Bhatti *et al.* (1996) added that the aqueous and alcoholic *T. foenum* seed extract is able to show a clear misalignment in positive and negative bacteria of gram stain and alkaloid, saponin and flavonoids compounds may lead to disruption of their function and cell death in addition to other compounds that affect on the respiratory chain of cells, which leads to a stop in the production of energy through the membranes and thus shows the deadly support for them toward the microorganisms (Cowan, 1999). *T. foenum* also contains phenolic compounds (Khuranu *et al.*, 1982) and this compounds act as an antimicrobial (Inderjit & Foy, 1999, Kocaca liskan *et al*., 2006) also *T. foenum* plant contains phenolic compounds that act as an antibiotic microscopy (Hussein and others, 2012), our results were close to those of Hussein and others (2012) who obtained highs inhibition value when treated *Staph. aureus* with *T. foenum* extract, also as consistent with the results of a study conducted before (Ghuson, 2015). Oribi (2017) pointed out that *O. sativum* works to disable pathogens, and that the containment of *O. majorana* plant extract flavonoids compounds has a widespread negative effect such as the antibacterial action (Ahn *et al*., 2007) as a result of an increase in the sensitivity of the cell membrane of bacteria and consequently a harmful occurrence in cell components and enzymatic reactions (Cuatlore *et al.*, 1997). Also, the presence of phenolic

compounds in the extract increases the sensitivity of the bacterial cell and affects the cell wall waste and the transport of electrons and other materials through the wall and changes in the manufacture of a section of cellular components such as nucleic acids and proteins as well as events inhibiting the action of some reactions and thus the negative impact on the reproduction bacteria and the end of its life cycle (Zangana, 2016), and *T. foenum* and *O. sativum* contain volatile oils that affect the growth of bacteria in different concentrations and the type of active compounds in its composition, as the essential oil contains phenolic compounds and turbines that affect the permeability of the cell membrane leading to a change in the transport of electrons, or changes in the composition of the cell membrane or the oxidation of oxidative enzymes, which leads to cell death (Zouari *et al*., 2010).

The effectiveness of the oregano extract is attributed to the fact that it contains a high amount of thymol compound that is effective in preventing the growth of microorganisms (Cosention *et al.*, 1999). Zangana and others (2016) and Al-Mashhadani (2017) added that *O. majorana* contain phenolic and thymol compounds that play an important role in weakening harmful bacterial colonies by breaking down the wall of harmful bacterial cells and clotting their protein and altering the permeability of its cytoplasmic membrane and thus outlining its vital processes, while Boskou and Lugouri (1996) indicated that the effectiveness of oregano plant in relation to microorganisms is due to its contain rosmarin compounds and volatile oils.

Table 6 : Effect of aqueous extract of *Trigonella foenum* and *Origanum majorana* on growth of isolated bacteria.

Plant / Bacteria	*Trigonella foenum*			*Origanum majorana*		
	Concentration(%)			Concentration(%)		
	25	**50**	**100**	**25**	**50**	**100**
Staph. aureus	9	13	33	9	12	33
Staph. epidermidis	6	10	28	6	11	26
Staph. saprophyticus	8	11	28	8	11	29
E. coli	5	7	19	6	8	24
P. aeruginosa	10	13	23	8	10	29

Table 6 : Effect of aqueous extract of *Trigonella foenum* and *Origanum majorana* on growth of isolated bacteria.

Plant / Bacteria	*Trigonella foenum*			*Origanum majorana*		
	Concentration(%)			Concentration(%)		
	25	**50**	**100**	**25**	**50**	**100**
Staph. aureus	10	13	35	10	12	35
Staph. epidermidis	7	12	30	6	8	28
Staph. saprophyticus	9	12	29	8	13	32
E. coli	6	8	23	6	9	24
Pseu. aeruginosa	9	15	28	9	13	30

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