



Study the effect of aqueous and alcoholic extracts of *Trigonella Foenum – Graececum* L and some antibiotics against some bacterial species isolated and identified from acne vulgaris infections for as ample of Sumer University students

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Abstract

Acne vulgaris is a chronic inflammatory disease of the Pilosebaceous unit (comprising the hair follicle, hair shaft, and sebaceous gland). The most common disorders treated by dermatologists and other health care providers while it most often affects adolescent, it is not uncommon adults and can also be seen in the children. The use of antibiotics for the treatment of disease such as acne and others, has led to high incidence of side effects, in addition to the emergence of resistant bacterial strains. In the present study, we used fenugreek (*Trigonella foenum – graecum* L) is a medical plant have an important inhibitory effects against bacteria. This plant is widely distributed. Throughout the world and which belongs to the family Fabaceae. Among (50) infection with acne, (28) were female and (22) male. This is a random result in this study, because, the numbers of female students in summer university is higher than male. The present study appeared the spreading of acne infection in earlier age of female than male. 36 specimens were gram – positive bacteria referred to staphylococcus (28) isolates were *S. epidermidis* and (10) isolates were *S. aureus*, (8) isolates were *Propionibacterium acne*. Also, (3) isolates yeasts referred to *Pityrosporum spp.*, and (1) isolates was gram – negative. *Aureus* and *S. epidermidis* were variable insensitivity against antibiotics. The higher sensitivity was against cephalic. For all two types of Bacteria in this study we note that the aqueous extract was more effective in the inhibition of bacterial growth than Alcohol extract.

Keywords: Acne vulgaris, *Trigonella foenum – graecum*, antibiotics

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INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit (comprising the hair follicle, hair shaft, and sebaceous gland), and is among the most common dermatological conditions worldwide, with an estimated 650 million people affected (White 1998). Acne is considered a chronic disease owing to its prolonged course, pattern of recurrence and relapse, and manifestation such as acute outbreaks or slow onset (American Academy of Dermatology 2016). Also, Acne is considered one of the most common disorders treated by dermatologists and other health care providers while it most often affects adolescent, it is not uncommon adults and can also be seen in the children (Jawetz et al. 2007). The use of antibiotics for the treatment of disease such as acne and others, has led to high incidence of side effects, in addition to the emergence of resistant bacterial strains. Herbal remedies Used in the traditional folk medicine provide

an interesting and still largely one explored source for the creation and development of potentially new drugs for chemotherapy (Ali et al. 2007). The medicinal and aromatic plant are used for the treatment of disease. Medicinal plants receive attention to research centers because of their special importance in safety of communities (Najhafi et al. 2010). The curative properties of medicinal plants are due to the presence various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan et al. 2009). Secondary metabolite grouped as alkaloids, glycosides, flavonoids, saponins, tannins and essential oil (Najafi 2013). In the present study, we used fenugreek (*Trigonella foenum – graecum* L) is a medical plant have an important inhibitory effects against bacteria. This plant is widely distributed.

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Throughout the world and which belongs to the family Fabaceae. Fenugreek. An annual legume, is extensively cultivated in most regions of the world for its medicinal value (Moradi kor et al. 2013). Fenugreek leaves and seeds consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic- lowering- blood sugar- and cholesterol level anti-cancer, antimicrobial, etc) Also, the seeds reduce sickness due to air pollution (Acharya et al. 2006). Some source of Fenugreek seeds have been reported to possess antimicrobial activity against both gram Positive and gram negative bacteria (Bhatti et al. 1996). The present paper describes the results of some bacterial species accompanying with acne infections, and study the effect of Fenugreek (*Trigonella foenum, graecum*) as an antibacterial.

MATERIALS AND METHODS

Fifty samples were isolated from some colleges of Sumer University, these are: college of Agriculture, college of Basic education and college of Administration & Economics. All students samples were infected by acne (22 male and 28 Female) in the range of age (18 – 24) year through the period 1/11/2017 to 1/4/2018.

Isolation & Identification

All samples were examined by microscope in addition of the direct examination for samples by making a smear of Gram stain for all samples – Then cultured by nutritional media, specialized with primary cultured there: Mack on key agar to gram – negative bacteria and Blood agar base to isolates gram – positive a Europhilic cocci bacteria. Then, the plates of Blood base agar were incubated anaerobically with the presence of Gas Back Jars to isolates p. acne bacteria at 37 c for 24 hrs. Mannitol agar were used for the differentiation between the species of staphylococcus spa. by Mannitol fermentation. Differentiated biochemical tests were used in addition of gram – stain test, and noting the shapes of colonies that growing on the nutritional media ,such as that growing on Blood base agar (Jawetz et al. 2007), Indole production (Macfagddin 2000), catalase production (Baron et al. 1998) and Mannitol fermentation (Betty et al. 1999).

Sensitivity Tests of Standard Antibiotics Disc Asses of Five Antibiotics

Sensitivity tests were used to detect the sensitivity of bacteria to antibiotics. Antibiotics were: cephalixin, Erythromycin, Ampicillin, Lincomycin, and penicillin (Bauer et al. 1966). After doing the identification, suspension of bacteria were prepared, Antibacterial activity was determined by disc diffusion method (Bauer et al. 1966). Muller- Hinton agar plates were inoculated with 0.2ml of bacterial suspension of overnight .culture of each bacterium adjusted to a 0.5 McFarland turbidity standard (108 colony forming units [cfu/ml]) and

uniformly spread out. The plates were incubated at 37 c for 24 hrs (Indu et al. 2006). After the incubation period, inhibition Zones were around the discs were measured and recorded, each test was carried out in trip locates. The growth inhibition diameter was an average of three measurements, taken at three different directions.

Preparation of Aqueous Extract of (*trigon ella foenum – graecum L.*)

The fresh plant of fenugreek (the famous species in Iraq was *trigon ella -foenum –graecum*) was collected from locally markets. Plants were identified by plant taxonomy laboratory in Basra –university –the leaves dried in shade so as to prevent the decomposition of chemical.

Constituents and was powdered by electric waring blender. 50 gram of plant powder was taken, then, 50ml of boiling distilled water were added to it (by 100 c). The mixture were filtered by three layers of gauze to separates the large format. After using the centrifuge for the final filtration (300 cir/min) to (15) min –this extract was considered as a basic concentration (100%). Then, a seral dilution of this extract were prepared: 5 %, 10%, 20%, 30%, 40% (Mohmmad 1995). Bacterial specimen were cultured on Muller – Hinton media, the well – diffusion method were used ,to detect the Anti –bacterial activity by making a well in (0.8) diameter of Muller – Hinton media that inoculated with bacterial, bacterial isolates, using a sterile purer 0.1 ml of the serial dilution extract were trans ported to wells, then incubated at 37c to 24 hrs. The inhibition zones around the wells were measured and recorded. Each test was carried out in triplicates. The growth inhibition diameter was an average of three measurements, taken at three different direction (Mahmood et al. 1989).

Preparation of Alcoholic Extract

Preparation of alcoholic extract powdered plant material was soaked in 250 ml of ethanol (95%) in ratio of 1:5 weight /volume in (60c-70c) for 6 hours. Extract was filtered and then a serial dilution. This extract were prepared: 5%, 10%, 20%, 30%, 40% (Shahab 2012). Well–diffusion method was used as described above in the preparation Aqueous extract. Plates were incubated at37c for 24 hrs. The inhibition zones were measured and recorded. Each test was carried out in triplication (Indu et al. 2006).

Antibacterial activity plant extracts were measured against only *staphylococcus epidermis* among all the bacterial isolates, because this species have the highest ratio of bacteria that isolated from the infested regions.

RESULTS & DISCUSSION

Among 50 infection with acne, 28 were female and 22 male. A randomly result in this study, because, the numbers of female students in Sumer University is higher than male. This may be interpretation to the female nature inquiry and medicinal consultation more

Table 1. Microorganisms which isolated from infectious region of acne

Microorganism	Total number of isolates
<i>Staph. Aureus</i>	10
Staph. epidermids	28
<i>P. acne</i>	8
<i>Pityrosporum spp</i>	3
Other bacteria	1
Aggregates of Micro organism isolates	50

Table 2. Inhibition zone in growth of *s. aureus* and *s. epidermids* by using some antibiotics

Antibiotics	<i>s. epidermids</i>	<i>s. aureus</i>
Cephaloxin	12 mm	10 mm
Erythromycin	10 mm	8 mm
Ampicillin	5 mm	6 mm
Lincomycin	6 mm	5 mm
Penicillin	5 mm	4 mm

Table 3. Inhibition zone by cause using of *Trigonella foenum* extracts (aqueous & Alcohol extract) against *s. epidermids*

Strain	Concentration of alcoholic extract/	inhibition zone mm	Concentration of aqueous extract	inhibition zone mm
<i>Staph. epide ermids</i>	5	4.5	5	2
	10	6	10	6
	20	7	20	8
	30	8	30	12
	40	12	40	16

than male. The present study appeared the spreading of acne infection in earlier age of female than male. This may be referred to the prematurely ripe of female than male (Babacan et al. 2015, Shahab 2012). This results agree with Farag (1989). The results showed in **Table 1** that microbial identification. 36 specimens were gram – positive bacteria referred to staphylococcus (28) isolates were *s. epidermidis* and (10) isolates were *s. aureus*, (8) isolates were *propionibacterium a. cne*. Also, (3) isolates yeasts referred to *pityrosporum spp.*, and (1) isolates was gram – negative.

Antibacterial Sensitivity of Antibiotics

Table 2 shows the effects of some antibiotics against the growth of *s. aureus* and *s. epidermids*. The antibiotics were: Ampicillin, Erythromycin, cephalixin, Lincomycin and penicillin. The most influential were (Bauer et al. 1966, Betty et al. 1999, Davoobadi and Aghajani 2013, Karthikeyan et al. 2009) mm for cephalixin, Erythromycin and Lincomycin in series. This results with regard to *s. epidermidis*. *s. aureus* sensitivity were: (10, 8, 6, 30) mm to cephalixin, Erythromycin and Ampicillin in series.

Table 2 explain that *staphylococcus* was variable in sensitivity to antibiotics. Generally, it was sensitive to almost of antibiotics. *S. epidermids* appears lower level of Sensitivity (high resistance) to Ampicillin and penicillin and higher level of sensitivity to cephalixin. *S. aureus* isolates appear high resistance to penicillin and low resistance to cephalixin. This may be referred to the bad and repetition utilization of this antibiotic, that cause to the appearance of new strain carried one gene or more encoded to Beta lactase enzymes that play an important role in the resistance of bacteria to this antibiotic. Sensitivity of two isolates to cephalixin may be interpreting to the little use of this antibiotic against

bacteria that led to not developing of bacteria to means resistance this antibiotic (Karthikeyan et al. 2009).

The result showed in **Table 3** that the aqueous extract were more effective in the inhibition of bacterial growth than Alcohol extract. This may be referred to the assurance of flavonoids in fenugreek that protects the mucoid layer of skin through the prevention of forming the lesions by different necrosis factors (Gollnick et al. 2008).

The most important effective compounds of fenugreek are: alkaloids and sterols. Alkaloid have killer effect of microorganisms because of their ability to the interference with cell DNA (Saure et al. 1996). This result agrees with Wagh et al. (2007). It most knowing that we tested the sensitivity of *s. epidermids* against Fenugreek extract because this bacteria showed the higher ratio among all the bacterial isolates accompanying with acne infection.

The present study concluded to the appearance of high ratio isolation for two type of Bacteria, these are: *s. epidermids* and *s. aureus* among the infection of acne, and the two types of isolates appear a high ratio of drug sensitivity against cephalixin. In this study, *s. epidermids* was selected for experimentation fenugreek extracts on him, because this species of bacteria have the higher ratio of isolation, *s. epidermids* showed high sensitivity against fenugreek extracts (Aqueous and alcoholic extracts values, so this study recommended to use fenugreek extracts as sources of antimicrobials with stable biologically active components that can establish a scientific base for the use of plants in modern medicine.

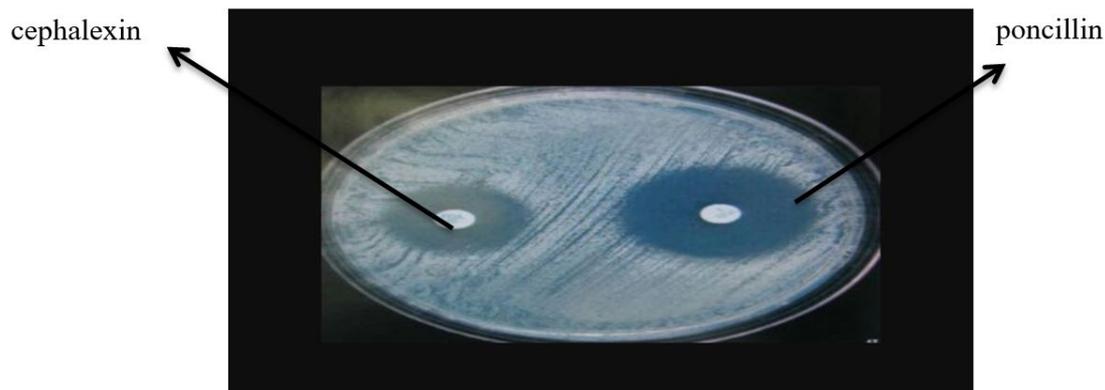


Fig. 1. Inhibition zones by cause using cephalalexin and penicillin against *s. aureus*

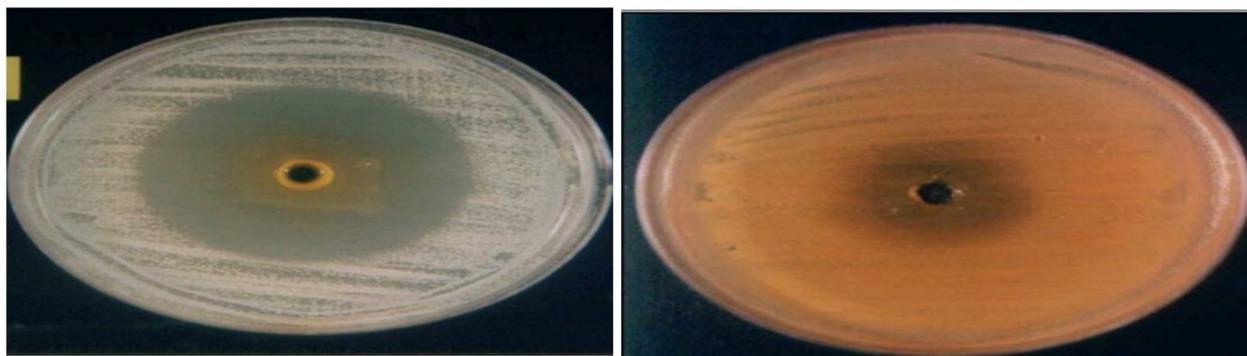


Fig. 2. Inhibition zones by cause effect of alcohol extract and aqueous extract against *s. aureus*

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