IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2016 Vol.:6, Issue:1

Antibacterial Activity of *Trigonella foenum- groecum* Essential Oil against Skin Infection with *Staphylococcus aureus*: *In Vitro* and *In Vivo* Studies

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Ibrahim Salih Abbas[3], Ahmed Rahma Abu-Raghif[1], Shurooq rayyis kadhim[2], Ahmed Hamza AJMII[6], Maysaa ali abdul khaleq[4], Kawther Abdul Mahdi Naji[5]

[1]*Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq*

[2]*Department of Laboratory of clinical science, College of Pharmacy, Al-Mustansyria University, Baghdad, Iraq*

[3] *Departments of Pharmacognacy, College of Pharmacy, Al-Mustansirya University, Baghdad, Iraq*

[4]*Department of pharmacology, College of pharmacy, Al-Mustansyria University, Baghdad, Iraq*

[5]*Departments of Dermatology, Al- Yarmook Teaching Hospital, Baghdad, Iraq*

[6]*The State company for Marketing Drugs and Medical Appliances / Ministry of Health, Baghdad, Iraq*

Submission: 2 April 2016
Accepted: 5 April 2016
Published: 25 April 2016

Keywords: Antiracial activity, *T. foenum- groecum*, *Staphylococcus aureus*

ABSTRACT

Antibacterial activity of *T. foenum- groecum* essential oil extract (1.2gm/100 µl) was investigated in multi- drug resistance (MDR) *Staphylococcus aureus* specimen isolated from patients with skin infection in Baghdad. *T. foenum-groecum* use externally for cellulites and skin inflammation due to the presence of diosgenin.fast liquid chromatography was used to separate these components. Antibiotics combinations revealed that *T. foenum- groecum* essential oil with Clindamycin against MDR isolates of *S. aureus* showed a synergistic effect when used as 1/4 MIC for each antimicrobial. *In vivo* study was executed to determine antibacterial activity of these compounds by induction of skin infection with *Staphylococcus aureus* in mice and the treatment begun after 4hrs later and continue to seven days then skin biopsy was taken and sent for histopathological examination. According to the results of this study, we can conclude that *T. foenum-groecum* essential oil has remarkable antistaphylococcal activity. Combination of *T. foenum-groecum* essential oil with Clindamycin was more effective than Clindamycin alone in treatment of skin infection with *Staphylococcus aureus*.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

Multi- drug resistant (MDR) *Staphylococcus aureus* infections have become a major public health concern in both hospital and community settings [1,2]. Since the late 1990s, community-associated MDR *Staphylococcus aureus* emerged as a principal cause of skin and soft tissue epidemics throughout the world [3]. It has been estimated that mortality rates from MDR infections in U.S. hospitals are higher than those from infections caused by HIV/AIDS and tuberculosis combined [4]. Furthermore, there is a considerable medical challenge in treating MDR infections due to the remarkable ability of *S. aureus* to develop resistance to multiple antibiotics, thus limiting the number of viable therapeutic options [2,5]. The resistant strains of *S. aureus* are often methicillin- resistant or nafcillin- resistant by virtue of changed penicillin-binding proteins. Therefore, there is an urgent need to develop novel antimicrobials with unique mechanisms of action to combat MRSA. The drug of choice for these *Staphylococci* is vancomycin, to which Gentamicin is occasionally added. Clindamycin or Trimethoprim- sulfamethoxazole can be used to treat non- life –threatening infections caused by these organisms[6]

T. foenum- groecum is one of the most widely used ingredient alternative medicine for the treatment of wounds [7]. According to the fact that about 30% of drugs used worldwide are based on natural products, because *T. foenum- groecum* has bioactive component with well known antibacterial activity (like diosgenin) which encouraged us to investigate the antibacterial activity of *T. foenum- groecum* essential oil against skin infection with *S. aureus* and to study probable synergistic activity in combination with Clindamycin.

MATERIALS AND METHODS

Isolation and detection of *Staphylococcus aureus*

All specimens were diagnosed microscopically (Gram stain), morphologically and biochemically according to standard methods[8,9], and biochemical tests using commercial kits (GP-VITEK2 Gram-positive colorimetric identification kit) for *Staphylococcus aureus* bacteria (BioM erieux, France).

Antibiotics susceptibility

Antibiotics susceptibility test was done by using the Biomérieux VITEK2 compact system (BioM erieux, France) against the following antibiotics: (aminoglycoside, floroquinolone, glycylcycline, mupripion, trimethoprim/ sulfamethaxazole, vancomycin, tecoplanin, and rifampicin, benzylpenicillin ,oxacillin, erythromycin, tetracycline and clindamycin).

Extraction of *T. foenum- groecum* essential oil

T. foenum- groecum essential oil is extracted from seeds of plant (supply from local markets in Baghdad). The oil was extracted by steam distillation from dried plant and accounted 1.2gm for *T. foenum- groecum* for each 100gm of plant materials[10].

Assessment of antibacterial activity of *T. foenum- groecum* essential oil

The antibacterial activity of essential oil was evaluated using agar well diffusion method with minor modifications [11]**.** The broth micro dilution method was used to determine minimum inhibitory concentration (MIC). All tests were performed in mueller hinton broth (Salucea (Netherlands) supplemented with Tween 80 (BDH (England) at a final concentration of 0.5% (v/ v) [12]**.**Fractional inhibitory concentration values (FICs) for antimicrobials combinations was used to determine the effect of antimicrobials combinations on multidrug resistance (MDR) strains of bacteria. FIC values used to assess the synergism between Clindamycin (Saudia Arabia) with *T. foenum- groecum* essential oil for *S. aureus*[13].

Separation of active ingredient of *T. foenum- groecum* essential oil

Separation of active ingredients of *T. foenum- groecum* essential oil was done by FSL (Fast Liquid Chromatography) (Shimadzu (North Amerika) equipped with binary delivery pump model 2010, using 3µ particle size column (50 × 4.6 mm H.D) C-18(Injection 10 µl of essential oil in column) , Mobile phase: 0.01M ammonium phosphate buffer (BDH(England) A: acetoitrile B (BDH(England). Eluted by linear gradient from 0-100% B in 10 min. Detection of eluted peak was monitored by UV-Vis spectrophotometer (Cecil (france) set at 254nm, flow rate 1.0 ml/min, temperature 30˚C [14]**.**

Animals experiment (*In vivo* method)

Thirty-five healthy, domestic male mice, weighing 23-25 grams were used in this study; they were obtained from animal house in high institute for infertility diagnosis and assisted reproductive technologies, in the period between August/2014 f dto October/ 2015. These mice were kept in separated cages; the room temperature was maintained at 20 -25˚C.

Animals grouping: The mice randomly divided into five groups (n=7, each) according to following:

Group 1(control): control group infected just by phosphate buffer saline (China).

Group 2(induction group): infected by bacteria without treatment.

Group3: treated with *T. foenum- groecum* essential oil alone for 7 days.

Group 4: treated with Clindamycin solution 1% (Saudia Arabia) alone for 7 days.

Group 5: treated with combination of Clindamycin solution and *T. foenum- groecum* essential oil for 7 days.

For preparation of inoculate, the bacteria were subcultured onto brain heart agar (BHA) (Oxoid (England) and incubated at 37°C overnight. Then one colony was inoculated into brain heart broth (BHB) (Oxoid (England) and incubated overnight at 37°C, the overnight culture was diluted 1:100 in fresh BHB and grown until the mid-exponential phase (approximately 3 hours). The bacteria washed twice and resuspended in sterile phosphate buffered saline (PBS)[15]**.** Before inoculation the mouse models of bacterial skin infection were sedated with ether. The flanks of the sedated mice were shaved with clippers when necessary and cleansed with an ethanol solution (BDH (England), and then make wound by scalpel cuts. The wounds were subsequently inoculated by 50 µl of the bacterial suspension. Then the mice were returned to their cages and observed. All mice had free access to food and water throughout the duration of the experiments. Animals were observed daily and skin lesion size, swelling, redness, amount of buss were noticed. The treatments with antimicrobial used in this study begin after 4 hrs of bacterial inoculation and continued for the regimens of 7 days [15,16].

Statistical Analysis

Data were analyzed using SPSS version 16 and Microsoft Office Word and Excel 2007. Nominal data were expressed as number and percent. Independent sample T-test was used for comparison of mean. *P*-value less than 0.05 were considered significant difference.

RESULTS

Out of 300 specimens obtained from different skin infection, 58 isolates (19.3%) were *S. aureus***.** Identification was performed using the commercially available identification Vitek2 GP card. *Staphylococcus aureus* specimens are highly susceptible to the most of antimicrobial agents (aminoglycoside, floroquinolone, glycylcycline, mupripion, trimethoprim/ sulfamethaxazole, vancomycin, tecoplanin, and rifampicin), and highly resistance to benzylpenicillin and oxacillin, while show moderate resistance to erythromycin, tetracycline and Clindamycin. The percentage of resistant isolates to each antibiotic is shown in figure (1).

Minimum inhibitory concentration of *T. foenum- groecum* essential oil for highly resistant *S. aureus* isolates (n=10) as followed: 5 isolates with MIC=1.2 gm/100µl, and 5 isolates with MIC= 0.6 gm/100µl (Table 1).while MIC of Clindamycin for isolates was determined using VITEK 2 AST method which represents <=0.25.

The effects of *T. foenum- groecum* essential oil alone and in combination with Clindamycin against *S. aureus* in different are shown in table (1)

The treatments with antimicrobial used in this study for *in vivo* study begin after 4 hrs of bacterial inoculation and continued to the regimens of 7 days. Then after seven days part of lesion area was tested for histopathological examination.

Group 1(control): the mice infected just by phosphate buffer saline, no lesion, no redness, no swelling, no death, and histopathological section showed normal skin without inflammatory cell infiltration, also no vascular congestion, no edema and no necrosis as showed in figure (2).

Group 2 (induction group): in this group the mice infected by *S. aureus* and left without treatment, skin lesion was occurred (1cm) with redness, swelling and pus, five mice dead in the first day. Under histopathological examination heavy infiltration of dermis and subcutaneous

tissue by acute inflammatory cell predominantly neutrophil, marked edema with proteinaceous exudates, and vascular congestion was shown as in figure (3).

Group 3 (*T. foenum- groecum*): healing began in third day and four mice died in second day. Histopathological examination showed mild inflammatory cell infiltration (neutrophil) of dermis and subcutaneous tissue, edema with mild vascular congestion as showed in figure (4).

Group 4 (Clindamycin): in this group pus, redness and lesion (1cm) remain until day seven and three mice died in second day. Histopathological examination showed thickness of epidermis, heavy infiltration of dermis and subcutaneous tissue by acute inflammatory cell with necrotic debris, extravagated red blood cell and pinkish oxidate (pus) as shown in figure (5).

Group 5 (*T. foenum- groecum* + Clindamycin**):** swelled and pus continued until day six but with less degree than when treated with Clindamycin alone, no death was occurred. Histopathological examination showed moderate edema, few inflammatory cell infiltrations and no vascular congestion as shown in figure (6).

DISCUSSION

In the present study, correct identification rate of *S. aureus* in this study was representing 100% (58/58) other study found that correct identification rates of *S. aureus* were 99.5% [17].In this study *S. aureus* isolate represent 19.3% of patients with skin and soft tissue infection which similar to study done in Ethiopia in which *S. aureus* represent 19 % of patient with infected wound[18]**.**

Zone of inhibition of *T. foenum- groecum* essential oil against *S. aureus* was ranging from 12 to 22mm of concentrated essential oil (100%), and some essential oil did not have any antibacterial effect against seven isolates. Other study mentioned that antibacterial activity of *T. foenum-groecum* essential oil against gram positive and gram negative bacteria have zone of inhibition above 7mm in diameter was considered as a positive result [19].

In this study, it was found that MIC of *T. foenum- groecum* essential oil ranging from 0.6-1.2 gm/100µl. Other investigations revealed that MIC values of *T. foenum- groecum* essential oil were ranging from 0.8 -6.4 gm/100µl against both gram positive and gram negative bacteria[19].

Susceptibility test was conducted for all *S. aureus* isolates against different antibiotics as following: to β- lactam antibiotics (benzyl penicillin MIC ≥5µg/ml and oxacillin MIC ≤0.25-≥4µg/ml) *S. aureus* isolates demonstrated a resistance rate reached to 100%, 86% respectively. Mean while all *S. aureus* isolates were sensitive to aminoglycosides group (gentamicin MIC ≤0.25- 1µg/ml and tobramycin MIC ≤1µg/ml), fluoroquinolones (levofloxacin MIC ≤0.12-≥2µg/ml and moxifloxacin MIC ≤0.25µg/ml), glycylcycline antibiotic (tigacyclin MIC ≤0.12-0.25µg/ml), mupripion MIC ≤2- ≥8µg/ml, and trimethoprim/ sulfamethaxazole MIC ≤10-≥20µg/ml (i.e 0% resistance for all antibiotics).

Three isolates of *S. aureus* (5%) in this study showed resistance to vancomycin**,** many studies demonstrate that among all tested antibiotics, vancomycin seems to be the most effective antibiotic for *S. aureus*[20], The susceptibility to this antibiotic regarded it as drug of choice to treat severe infection caused by *S. aureus* that resistance to methicillin and other β- lactam antibiotics.

Nair *et al* (2013)[21] found that resistance rate for rifampicin and Clindamycin reach up to 2.7%, 1.4% respectively which agree with the result of present study. Other study showed that resistance of *S.aureus* to Clindamycin was 20.3%[22]**.** However, widespread use of Clindamycin antibiotic has led to an increase in the number of Staphylococcal strains acquiring resistance to it [23].

In this study, MDR isolates represent 81% of *S. aureus* tested strains. Those strains were resistant to all of the agents in two or more classes of antibiotic [25]. The result of present study was agreement Nair *et al*. (2013)[22] who found that MDR *S. aureus* was 78%.

Synergistic effect was seen from combination of Clindamycin with *T. foenum-groecum* essential oil against most *S. aureus* isolates as shown in table (1)

The growth of the organism was clearly observed in all inoculated mice. Lesions cultures was confirmed the infections by bacteria. After usage of the plants as topical treatment for one week, the lesions and wounds were healed dramatically. Control groups were used to prove that healing was not spontaneously.

In recent years, different reports from different countries were indicated that there were antimicrobial activities of medicinal plants, for many years, the effect of herbal medicine on burn

wound has been noted. Herbal products seem to possess moderate efficacy and are less expensive as compared with synthetic drugs. Many plants and plants-derived products have been shown to possess potent wound-healing activity [26].

In-vivo-sensitivity of the plants studied on the infected mice proved to be very active. All the infected mice were cured by local application of the *T. foenum-groecum* on the lesions. No spontaneous improvement was detected on the infected control mice. The result of the histopathological examination in the present studies showed that antibacterial activity of *T. foenum- groecum* essential oil alone and in combination with Clindamycin was greater than antibacterial activity of Clindamycin alone. This effect may belong to the active compound in the *T. foenum- groecum* which have a bactericidal effect against *S. aureus*. The use of that *T. foenum- groecum* in the form of topical therapy in infected mice was proved the affectivity of *T. foenum- groecum* plants as medicinal purpose[21].

Most of the medicines are mixture of many plants, but none of these traditional ointments were scientifically studied. In the current study, *T. foenum- groecum* extract was compared with Clindamycin as the standard treatment for burn wounds in mice. The actual mechanism of improved healing is still unclear, the probable mechanism is providing necessary material for healing, increasing blood flow to burn area, decreased an inflammatory response, and decreasing the rate of infection. A new skin medication can be introduced by usage of herbal medicines with fewer adverse effects and shorten the period of healing thus decrease the rate of hypertrophic scar. The result findings denotes of *T. foenum- groecum* in healing of burn injuries as an inexpensive and available herbal medicine[25].

CONCLUSION

T. foenum- groecum essential oil has antibacterial effect against skin infection with *Staphylococcus aureus* and combination of *T. foenum- groecum* with Clindamycin shows synergistic effect and is more effective than Clindamycin alone.

Note: the research abstracted from Ph.D. thesis

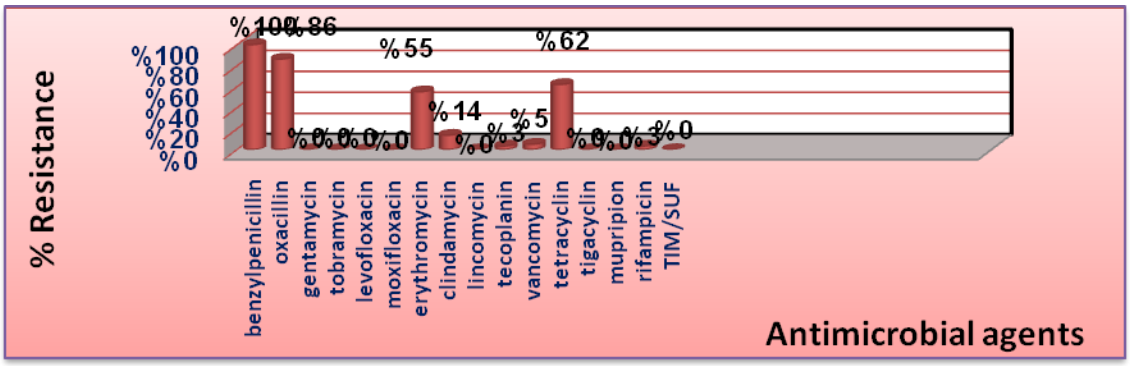


Figure (1): Resistant *S. aureus* isolates to antimicrobial agents (n=58)

Table (1): Effect of *T. foenum- groecum* essential oils and Clindamycin alone and in combination with each other on MDR *S. aureus* isolates (n=10)

Isolates No.	*T. foenum-groecum* 1/4 MIC	*T. foenum* + clindamycin		FIC		Clindamycin 1/4 MIC
		1/4 +1/4 MIC	1/2 +1/2 MIC	Values	interpretation	
1	-	-	-	0.5	Syn.	+
2	+	-	-	0.5	Syn.	+
3	-	-	-	0.5	Syn.	+
4	-	-	-	0.5	Syn.	+
5	-	-	-	0.5	Syn.	+
6	+	-	-	0.5	Syn.	+
7	+	-	-	0.5	Syn.	+
8	-	-	-	0.5	Syn.	+
9	+	-	-	0.5	Syn.	+
10	-	-	-	0.5	Syn.	+
Mean ± SD	215 mg ± 74.7	2.15 mg ±74.7				0.0008 mg ± 0.001
P-Value	0.470	0.470				

*Where – mean no growth while + mean growth. The fractional inhibitory concentration (FIC) was determine as follow: (≤0.5) synergism, (0.5–<1) additive, (1–<4) indifference,(≥4)antagonism. *P*-value less than 0.05 were considered significant.

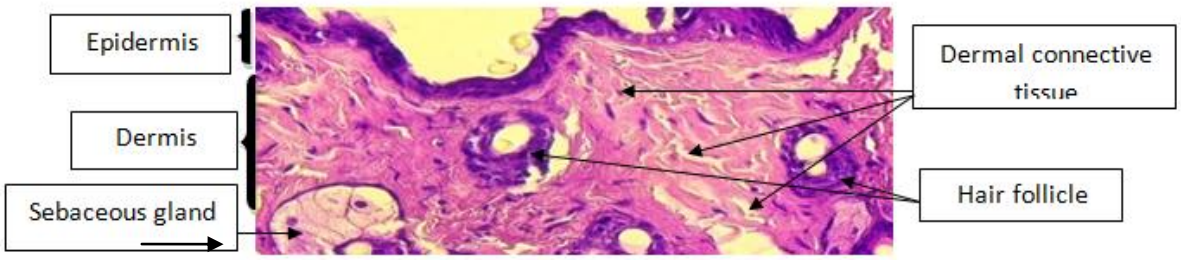


Figure (2): Section of mouse infected by phosphate buffer saline (H×E) in 40×- Power

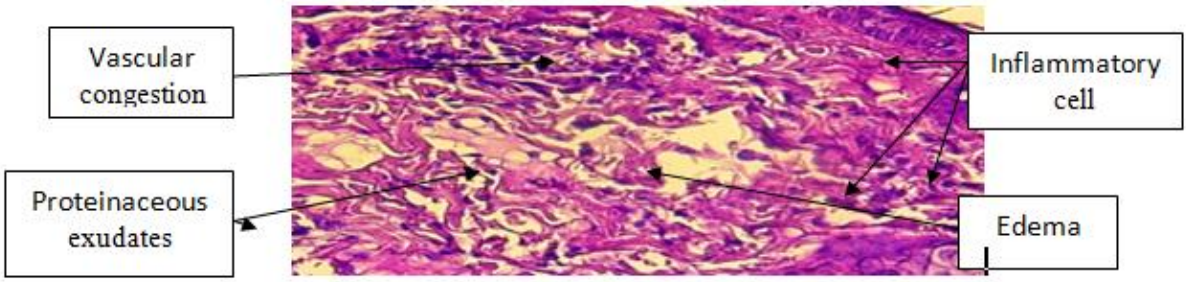


Figure (3): Section of mouse infected by *S. aureus* without treatment (H×E) in 40× Power

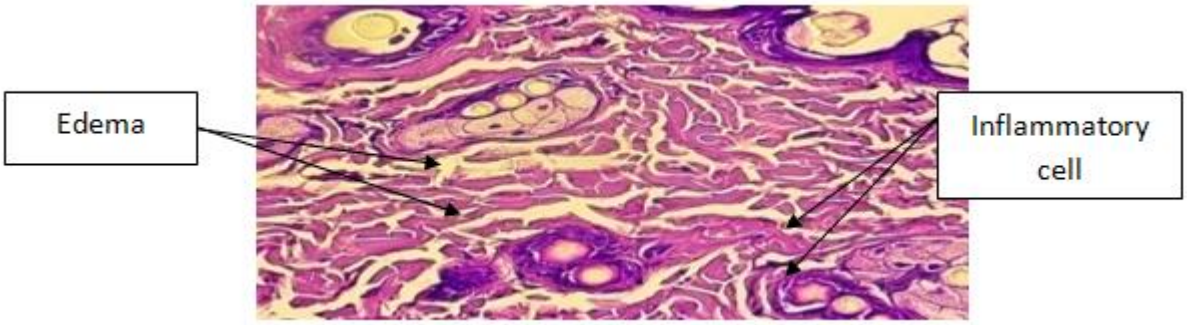


Figure (4): Section of skin specimen of mouse infected by *S. aureus* treated with *T. foenum-groecum* (H×E) (40×)

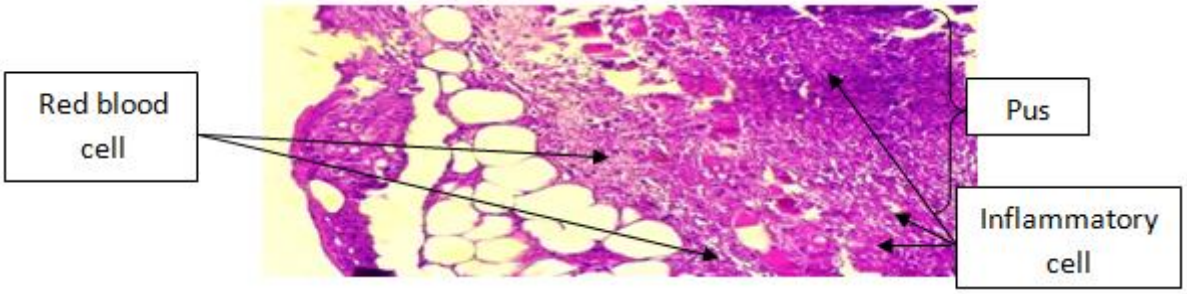


Figure (5): Section of mouse infected by *S. aureus* treated with Clindamycin (H×E) in 40×-Power

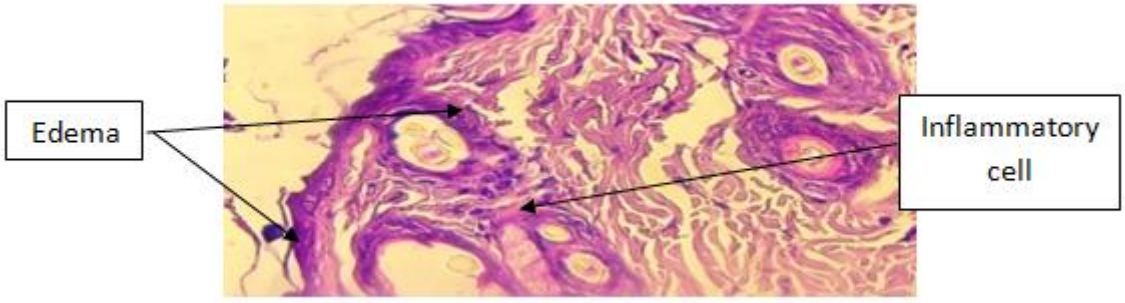
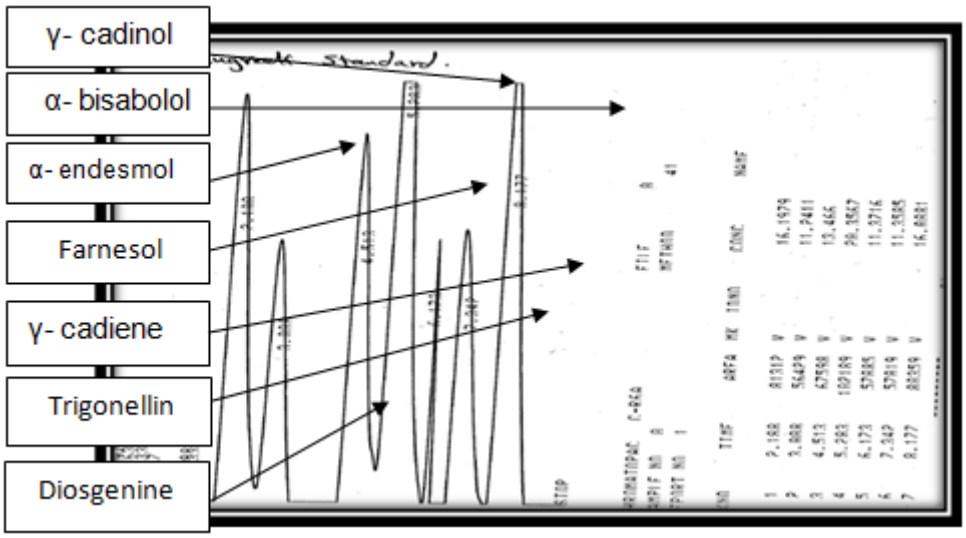


Figure (6): Section of skin specimen of mouse infected by *S. aureus* treated with combination of *T. foenum- groecum* and Clindamycin (H&E) (40×)

(B1)



(B2)

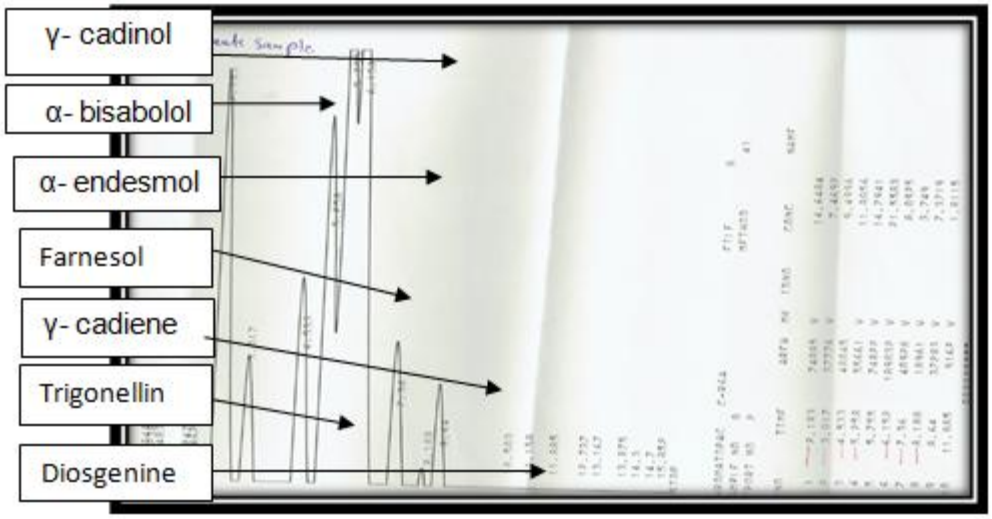


Figure (7): HPLC chromatography of fenugreek standard and sample, B1: fenugreek standard, B2: fenugreek sample.

REFERENCES

1.Naimi TS, LeDell KH, ComoSabetti K, Borchardt SM, Boxrud DJ, Etienne J,Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, and Lynfield R. "Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection". 2003, JAMA 290:2976–2984. doi:10.1001/jama.290.22.2976.

2. Tsuji BT, Rybak MJ, Cheung CM, Amjad M,and Kaatz GW. "Community- and health care-associated methicillin-resistant *Staphylococcus aureus*: a comparison of molecular epidemiology and antimicrobial activities of various agents". Diagn. Microbiol. Infect. Dis. 58, 2007, 41–47.doi:10.1016/j.diagmicrobio.2006.10.021.

3. Stryjewski ME, and Chambers HF. "Skin and soft-tissue infections caused by community-acquired methicillin-resistant *Staphylococcus aureus*". Clin. Infect. Dis. 46(Suppl 5), 2008, S368–S377. doi:10.1086/533593.

4. Boucher HW, and Corey GR. "Epidemiology of methicillin-resistant *Staphylococcus aureus*". Clin. Infect. Dis. 46(Suppl 5), 2008, S344–S349.doi:10.1086/533590.

5. Chambers HF, and Deleo FR. "Waves of resistance: Staphylococcus aureus in the antibiotic era". Nat. Rev. Microbiol. 7, 2009, 629–641. doi:10.1038/nrmicro2200.

6- Warren Levinson MD. "Review of Medical Microbiology and Immunology', 12th edition, Mc-Graw-Hill-Newyork,2012, pp. 109-117,.

7- Bruneton J." Pharmacognosy, Phytochemistry". Medicinal plants, Lavoisiler Publishing Co., France, 1995, 265-380.

8- Collee J.G.; Fraser A.G.; Marmion B.P.; and Simmons A. "Mackie and McCartney practical medical microbiology". 14th edition: Churchill Livingstone, London, 1996, pp. 14.

9- Green wood O.; Slack R.; and Penther J. "Medical Microbiology". 15th edition, Churchill Livingstone, London, 1997.

10- Walton and Brown (1999): Chemical from plants, Imperial College Press.

11- Dahiya P.; and Purkayastha S. "Phytochemical analysis and antibacterial efficacy of Dill seed oil against Multi-drug resistant clinical isolates". Asian Journal of pharmaceutical and clinical research. Academic sciences, Issue 2, Vol. 5, 2012, issn 0974-2441.

12- Clinical and Laboratory standards Institute/ National comitee for clinical laboratory standards (CSLI/NCCLs). "Performance standards for antimicrobial susceptibility testing". Fifteenth information supplement, Wayne, PA.,2005 CLSI/NCCLS document M100-S15,

13- Levinson W.; and Jawetz E. "Medical Microbiology and Immunology", Examination and board review 5th edition, Lang Medical Books/ Mc-Graw-Hill, Newyork, 1998

14- Subbiah rajasekaran *et al*. "Antioxidant effect of Aloe vera gel in Streptozocin- induced diabetics in rat".pharmacological reports, 57, 2006, 90-96.

15- Cho JS.; Zussman J.; Donegan NP.; Romos RI.; Garcia NC.; Uslan DZ.; Iwakura Y.; Simon SI.; Cheung AL.; Modlin RL.; Kim J.; and Miller LS. "Non invasive in vivo imaging to evaluate immune responses and antimicrobial therapy against *Staphylococcus aureus* and USA 300 MRSA skin infections". J. Invest. Dermatol. 131,2011,907-915.

16- Fang RC.; Kryger ZB.; Buck DW.; Dela Garza M.; Galiano RD.; and Mustoe TA. "Limitations of the db/db mouse in translational wound healing research: is the 10 Polygenic mouse model superior? Wound". Repair Regen, 18, 2010, 605-613.

17- Edward J.P. Cartwright; Gavin K. Paterson; Kathy E. Raven; Ewan M. Harrison; Theodore Gouliouris; Angela Kearns; Brune Pinchon; Giles Edwards; Robert L. Skov; Anders R. Larsen; Mark A. Holmes; Julian Parkhill; Sharon J. Peacock; and M. Estee Török ."Use of Vitek 2 antimicrobial susceptibility profile to identify mec C. in Methicillin- resistant *Staphylococcus aureus*". J. Clin. Microbial., 51(8), 2013,2732-2734.

18- Girma Godebo; Geber Kibru; and Himanot Tassew. "Multi- drug- resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia". Annals of clinical microbiology and antimicrobials. No. 17(12), 2013, doi:10. 1186/1476-0711-12-17.

19- Manal M. Ramadan; H.A. Yehia; Mohameds Shaheen; and M. Sh. Abed El- Fattah (2014): Aroma volatiles, Antibacterial, Antifungal and Antioxidant properties of essential oils obtained from some spices widely consumed in Egypt. American-Eurasian. *J. Agric. and Environ. Sci*., 14(6): 486-494.

20- Fatemeh Nejatzadel- Barandozi ."Antibacterial activities and antioxidant capacity of Aloe vera". Nejatzadeh-Barandozi Organic and Medicinal Chemistry Letters, 3, 2013,5.

21- Babakir- Mina M.; Othman N.; Najmuldeen HH.; Norri CK.; Fatah CF.; Perno CF.; and Ciotti M. "Antibiotic susceptibility of Vancomycin and Nitrofurantoin in *Staphylococcus aureus* isolated from burnt patients in Sulaimaniyah, Iraq Kurdistan". New Microbiol, 35(4), 2012, 439-46.

22- Nair Kaur; R. Prasad; and A. Varma. " Antibiotic resistance among clinical isolates of *Staphylococcus aureus* and usefulness of antibiogram". Int. Journal Pharm. Bio. Sci.. 4(1), 2013, (B)957-964, ISSN 0975-6299.

23- Lt. Col Mahima Lall; and Bring A.K. Sahni. "Prevalence of inducible Clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples". Medical Journal Armed Forces INDIA. 70, 2014, 43-47.

24- Gadepalli R.; Dhawan B.; Mohanty S.; Kapil A.; Das BK.; and Chaudhry R. "Inducible Clindamycin resistance in clinical isolates of *Staphylococcus aureus*". Indian J. Med. Res., 123, 2006,571-3.

25- Saiman L. "Clinical utility of synergy testing for multidrug-resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis". Paediatr. Respir.Rev. 8, 2007, 249–255.

26- Hosseini SV.; Niknahad D.; Fakhar N.; Rezaianzadeh A.; and Mehrabani D. "The healing effect of honey, putty, vitriol and other herb in *Pseudomonas aeruginosa* infected burns in experimental rat model". Asian J. Anim. Vet. Adv., 6, 2011,572-579.