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Comparative study of Efficacy of Fenugreek oil and Levofloxacin against wound contamination by Staphylococcus epidermidis

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Abstract: ***Background****:* This study screened Where the forms of resistance varied in (*Staphylococcus epidermidis*) which is Recently, the dangers of residency have increased. ***Materials and methods****:* Where a sensitive antibacterial antibiotic (Levofloxacin), was selected compared with the oil evaluation of the antibacterial activity of Fenugreek essential oil via ***Results****:* MIC technique with concentrations between (500, 1000, and 2000 µg\ml) while Levofloxacin ranged between (0.5,1and 2) mg/ml. Histological results showed of *S.epidermidis* It showed changes in the skin infection from suppuration and abscess. Levofloxacin has a clear effectiveness for the treatment of skin ulcers, while the oil is superior in terms of skin healing. ***Conclusions****:* The goal result showed, that the infected with *S.epidermidis* after treatment locally by using Marjoram oil the skin regeneration and the healing process compared with Levofloxacin. The inconclusion result showed the effectiveness of oil antimicrobial agents *in vitro* and *in vivo*.

Keywords: *S.epidermidis*, Fenugreek oil, wound

INTRODUCTION

Common opportunistic and commensals Staphylococci are pathogens mainly found on the skin of humans and also in companion animals and domestic [1]. Life-threatening bacteremia from Staphylococci is the Diseases that take advantage of a person's weak immunity to *Staphylococcus epidermidis* and *Staphylococcus aureus* [2]. Life-threatening bacteremia from Staphylococci are a diverse group of bacteria that cause minor infections skin. The two major opportunistic pathogens of this genus are *S. epidermidis* and *S aureus* [3]. *S.epidemidis* is known to cause skin numerous including (acne vulgaris), which is youth One of the most common diseases on the face, papules, and pustules, upper back, and chest [4]. Erythromycin and Clindamycin are used for inflammatory acne treatment and the number of clindamycin-resistant strains is increasing [5]. Fenugreek (Trigonella foenum-graecum) is one of the esoteric culinary adjuncts that is used to improve color and flavor. It also alters the texture of food. In many traditional systems, this seed spice is also used medicinally as a galactagogue, antidiabetic agent, anorexia-fighting agent, gastrointestinal stimulant, and antibacterial. Numerous physiological benefits of fenugreek seeds have been observed in recent decades in both human and animal research [6]. The oil plant is used for different medicinal and used as a garnish in different countries. Also, different activities pharmacological of sweet marjoram [7]. The process of wound healing depends on many steps which, are inflammation and granulation of tissue,

matrix formation, and acquisition of wound strength [8]. The plant is used as a medicament for wound healing and its growing popularity is attributed to its easy availability, cost-effectiveness, and paucity of reported adverse reactions. The plant Majoronahortensis belonging to the family Labiateae possesses anti-cancer, anti-oxidant, anti-septic, and expectorant properties and was recommended for external applications in bruises, wounds, and skin infections [9]. The aim was natural oil as an alternative to medicines in the treatment of infections and wounds in laboratory animals.

MATERIALS AND METHODS

Samples Collection

Personalized isolates were obtained of swabs from skin isolated from the College of Science (Biology department)- University of Baghdad- Iraq.

Cultivation of Bacteria

At first, each sample was cultured on nutrient broth for activation after that media (Mannitol salt agar, blood agar, Staph 110, and milk agar) were used for differentiation of (*S.epidemidis*). All the isolates were incubated at 37 °C for 24 hours, and then identified by standard biochemical tests [10]**.** Biochemical characterizations of isolates were done by oxidase test, Catalase test, and coagulase test [11].

Plant Material

The plant was collected from the gardens of the -College of Science, dries well and fine grinding were performed, with 300g dry plant powder in 1.5 L distilled water for 5h. the volatile oil was extracted by hydrodistillation using Clevenger apparatus, and where it comes in concentrations between 500, 1000, and 2000 µg\ml was prepared with D.W (v/v) and kept at 4°C until use.

Dilution assay MICs

Determined were according to [12]. Concentrations of antibiotic were made and Levofloxacin causes MDR (Multi-drug resistance) between (0.5,1, 2) mg/liter while Fenugreek oil (500, 1000, and 2000 µg/ml).

Antibacterial Activity of Marjoram oil

The Fenugreek oil essential oil range (500, 1000, and 2000 µg/ml) was Attended by dissolving (50000, 100000, and 200000 ml.) from the extracted Marjoram essential oil in 100 ml. DMSO takes into consideration the volume of media to prepare the tested concentrations (500, 1000, and 2000 µg/ml) subsequently the assay of all concentrations by Agar Well Diffusion Method [13].

Experimental design

study was approved by the college's animal ethics committee and involved twenty male albino mice weighing 23±2 grams. The mice were separated into five groups for the experiment (n = 5 per group), and each group was given intraperitoneal xylazine (5 mg/kg) to induce anesthesia. To finish the experiment, the right flank was shaved with a disposable hand shaver after the hair was removed. Following that, a sterile lancet was used to create three parallel lines of superficial skin wounds, and the wounded skin from the second, third, and fourth infections with S. epidermidis was swapped out.

In positive control group mice in the 1st group were considered and the injured skin while also the *S.epidermidis* infected skin in the 2nd group without treatment. The third group was treated with antibiotics at 0.5 mg/ml after one day post-infection and the fourth group was treated with Marjoram oil (1000 µg/ml) both group treatments repeated every 12 hr for 3 days.

Histopathological Study

The samples were processed routinely and sectioned by microtome and the slides were stained by Hematoxyline and Eosin stain [14].

RESULT

In this study, the distribution of this disease among patient ages was studied in college. Most cases were in patients ages (18-29) years old in both sexes. Furthermore, the distribution of acne was higher among females than males (Table 1).

Table 1: S.epidermidis and S. aureus infection depended on age and Gender groups.

Bacterial Isolates Total NO. Of Cases		**Gender**		***Chi-Square* (χ^2) (P-value)**
		Male Positive No	**Female Positive NO**	
Staphylococcus epidermidis	13	5(38.46%)	8(61.53%)	10.438 ** (0.0001)

Isolation &Identification of Gram-positive (*Staphylococcus epidermidis*)

The microscopic examination of the bacteria was cultured and showed that cocci appeared as single cell pairs and chains these descriptions.

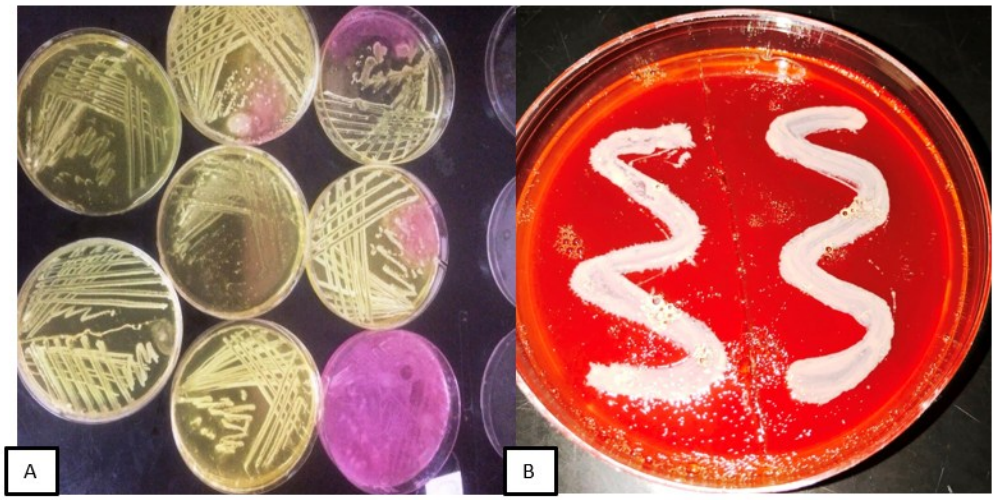

Figure 1: *Staphylococcus epidermidis*. *A: MSA,* B: Blood BSase Agar

Results show *S. epidermidis* Where the results showed its resistance to most of the antibiotics compared to index (CLSI) (Figure 2) and (Table 1).

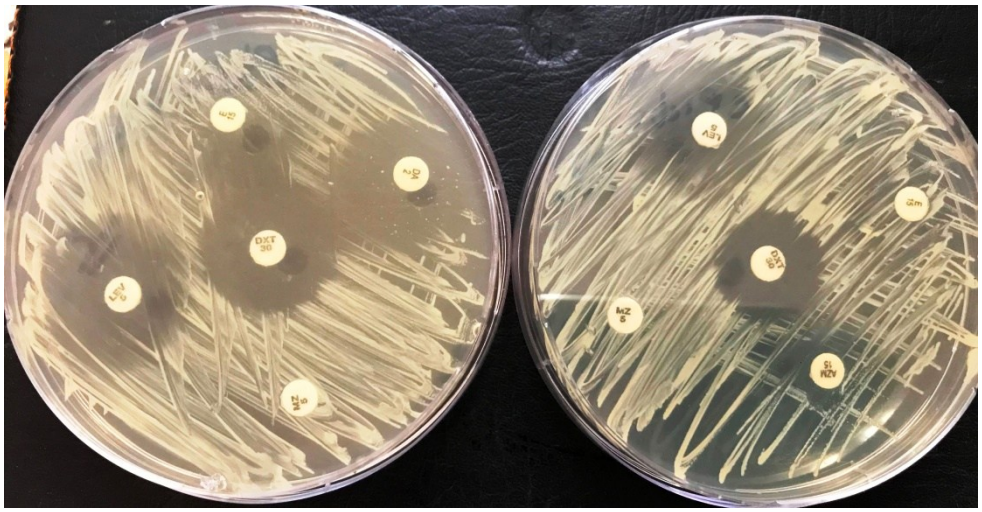

Fig 2: Antibiotic susceptibility test *Staphylococcus epidermidis*.

Table 2: Antibiotic suitability test

Antibiotic	***Staphylococcus epidermidis***
Methicillin	8 (61.53%)
Gentamycin	11(84.61%)
Chloramphenicol	6(46.15%)

Penicillin G	7(53.84%)
Vancomycin	4(30.76%)
Chi-Square (χ^2)	11.762 **
P-value	0.0001
** (P<0.01).	

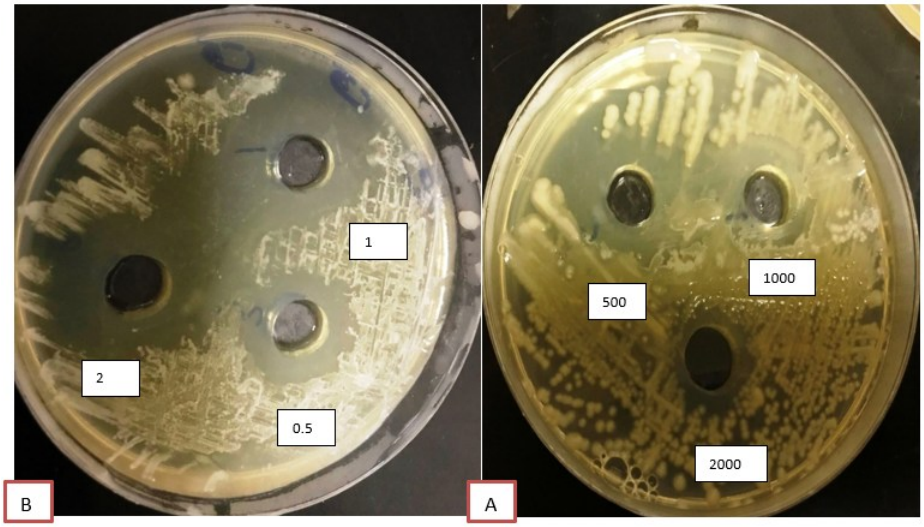

Figure (3): Comparison between MIC A) Fenugreek oil B) Levofloxacin against *S. epidermidis* on M H A plates at 37 ºC for 24 hours

Injury only skin at the first group the skin cells began to return to normal with the passage of time a cellular debris and inflammatory cells (Fig4 A), also in the dermis layer, few infiltrations of neutrophils and the epidermis layer hyperplasia (Figure 4 B).

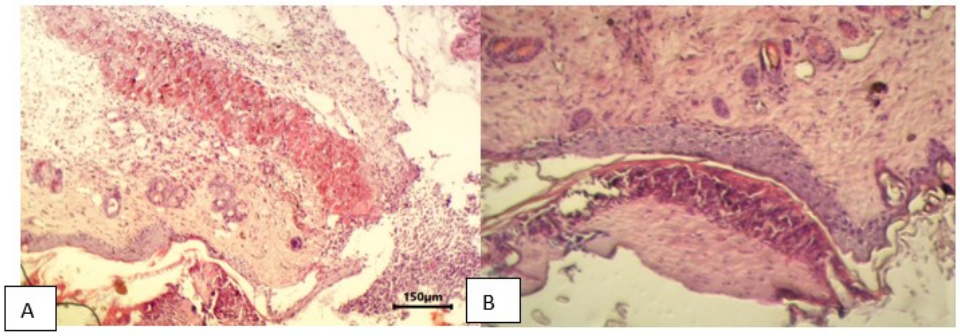

Figure 4: The skin of the 1st group (A) regeneration of the epithelial layer (B) hyperplasia of the epidermis layer

While infection with a bacterial isolate the 2nd group (injury + infection) after showed necrosis in all layers of the skin (Figure 5 A), While another section showed necrosis under the muscle layer (Figure 5B).

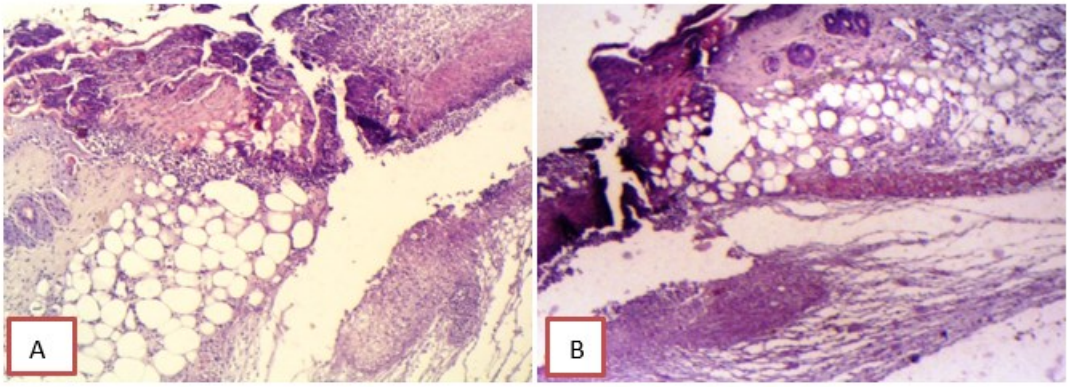

Figure 5: The 2nd group in the skin: (A) necrosis all layers of the skin B) necrosis under the muscle layer

The skin in the 3rd group (treated with Levofloxacin) regeneration of epidermal cells (Fig 6 A) In another section, there was a regeneration of skin cells and the production of collagen cells to produce new cells that help in regeneration (Figure 6 B)

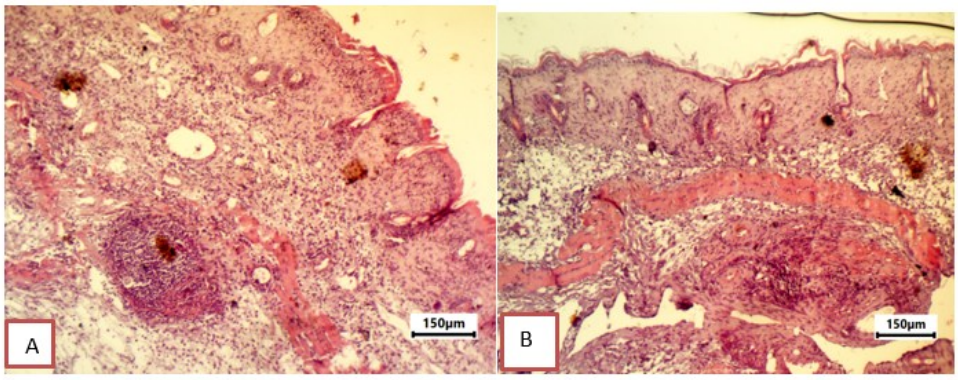

Figure 6: Histological in the skin of the 3rd group (A) mild regeneration and infiltration of dermis; (B) Regeneration in dermis by collagenous fiber

The last group (treated with **Fenugreek oil**) showed early healing signs and regenerated epithelial cells. One of the early signs of skin regeneration is the occurrence of a change in the epithelial layer and the return of the skin to its previous position (Figure 7), other sections clear complete regenerated epidermal epithelia.

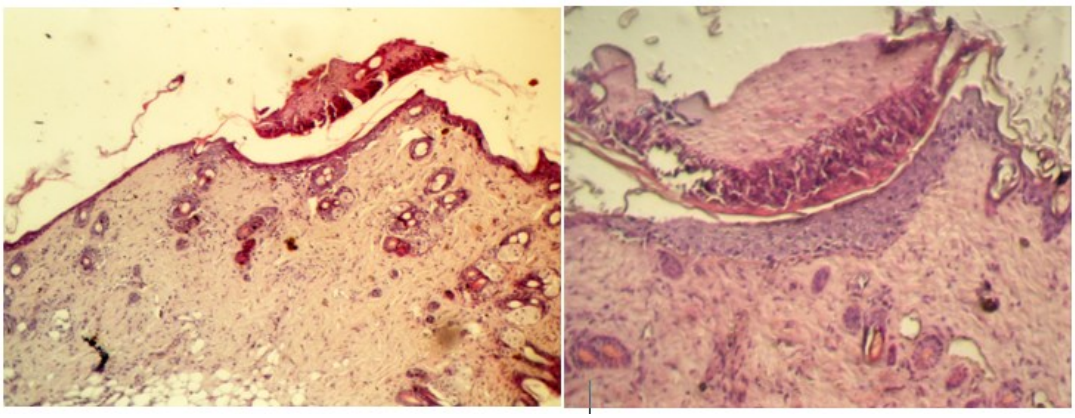

Figure 7: The 4th grouthe skin regenerated epithelial thick complete regenerated epidermal epithelia under the cellular debris (×400).

DISCUSSION

These results agree with Presnell *et al.* and Handke *et al.*, who shows gram positive, anaerobic bacterium, while nonpore resistant *S.* epidermidis culture of the follicular material [15, 16]. While This result by AlSaadi *et al.* shows most isolates of *S.aureus* were multi-resistance for antibiotics with a high level against Methicillin, Penicillin G, Methicillin and sensitivity to Vancomycin [17]. The 1st group as this stage represented the return of the skin to a normal condition without treatment. His agreement came with the occurrence of a recurrence of the skin after three days which is descried by AlSaadi *et al.* and He Z *et al.* [18, 19]. While the results of another researcher were the return of the skin to its position after 48 hours described by Ahmed *et al.* [20]. In the 2nd group virulent factor of bacteria causes sever tissue necrosis and neutrophils infiltration abscess formation may be due *S. epidermidis*, where this strain has virulence factors that make it resistant and difficult to treat [21]. The results agree with Marja *et al.*, who found the method that alcoholic extracts of *O. majorana* reduce micronucleus, total aberrant cells and different types of chromosomal fragment, ring chromosome [22, 23]. Levofloxacin (3rd group) treatment of the diseased skin caused tissue to exhibit symptoms of healing, although they were insufficient. However, when compared to oil Ahmed *et al.* the bacterium's high virulence or resistance to the antibiotic's action, antibiotic treatment did not demonstrate an adequate recovery rate because *S. epidermidis* outer membrane proteins [24, 25]. The 4th group (treated with Marjoram oil) showed typical healing sings compared with antibiotic It showed a slow progression with treatment, and this is due to the fact that these bacteria carry high resistance and high virulence [26]. Also, Campbell *et al.* provided the ability of topical application of Marjoram oil to reduce inflammation, during skin healing [27].

CONCLUSION

Recently, it became clear that there was severe resistance to antibiotics as a treatment in wound healing, and this is due to the fact that bacteria possess high virulence and severe pathogenicity. Alternative medicine is the use of vegetable oils instead.

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Conflict of interest: None declared

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