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Research Article

Effects of Bacterocin from MRSA and Nigella Sativa (seed oil) against Biofilm from MRSA

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ABSTRACT

A many risk challenge in (settings hospital) are multi- bacteria are antibiotic-resistant. Some type strains that ability adhesion surface-attached bio-film census. Fifteen MRSA isolates were considered as high biofilm producers Moreover all MRSA isolates; M3, M5, M7 and M11 produced biofilms but the thickest biofilm seen M7strain. The MIC values of *N. sativa* oil against clinical isolates of MRSA were between (0.25, 0.5, 0.75, 1.0) µg/ml While MRSAcin (50, 75, 100, 125) µg\ ml. All biofilms treated with MRSAcin and *Nigella sativa* developed a presence of live cells after cultured on plate agar with inhibition zone between MIC (18 – 15) and (14- 11)mm respectively.Yet, results showed that MRSA supernatant developed a inhibitory effect then habitat oil . Significant differences (P<0.05) were found in O.D. and viable count between pre and post treatment of M7 strain biofilms. Unlike the Nigella oil treatment which left live some bacterial cells, MRSA supernatant (MRSAcin) left no live cells.

Key words: Biofilm, MRSAcin, MRSA.

INTRODUCTION

Biofilm formation studies numerous have to in food-assioassion bacteria. Attachment plays a role in all the stages of biofilm formation including the initial maturation of the biofilm. Biofilms on food surfaces are a major problem leading to contamination spoilage of hazards (food and human health)[1]. Inhibition Compounds or interfering with (attachment bacterial and biofilm) are importance generation food preservatives as anovel class antimicrobial agents. Biofilm reducing target (the virulence mechanism) of the bacteria no inhibiting its growth. Hence, the chances of development reducing of resistance as no selective exerted on the pathogencity[2]. Mortality around the world cause of third significant diseases as estimated by World Health Organization. Infections by Methicillin-Resistants have become most health problem especially in hospital by causing skin infections simple to infections life-threatening[3]. *Staphylococcus aureus* is a Gve+ bacterium and is known to develop resistance quickly to antimicrobial agents. MRSA usually cause community acquired infections post-operative, endocarditis, TSS, poisoning food and osteomyelitis[4]. Drug-resistant pathogens incidence increasing drawn the attention of the pharmaceutical on the commercial traditional medicine in different countries, the using of drugs derived from plants have accelerated in recent years[5]. Plant medicinal like *Nigella sativa* is a widely used throughout the world. Seeds and oil have a long history of medicines and food usage in various systems. The broadly used seeds of *N. sativa* have been in the treatment of different diseases and ailments[6]. *N. sativa* has got the placepower of healing, among the top evidence based medicines herbal. Thymoquinoneit has a potent bioactive which shows promise in treating allergies and the immune system[7]. Seed of *Nigella sativa* is widely used in Iran out of the medicinal plants, herbal oil which is native to southwest Asia and has a rich historical and cultural heritage, due to a wide range of medicinal properties[8]. They called is Kalonji, *N. sativa* in South Asia, in Arabic as (Habbat-uL-Sauda), and commonly referred as black (cumin) in English[9]. Therefore, the present study was conducted to evaluate the anti-MRSA activity of *Nigella sativa* seeds using appropriate methods and to formulate a suitable topical gel formulation for its anti-acne activity.

MATERIALS AND METHODS

Specimen's collections of (MRSA), Fifteen specimens included: (Nasal swab) were collected from Al-Yarmouk hospital. All swab culture directly on Mannitol salt and Blood agar plates, the specimens were transferred at present to the microbiology laboratory isolation of bacterial pathogens and incubated at 37^0C for 24 hrs then the isolated colonies were identified on the basis of morphological, cultural and biochemical characteristics[10]. For screening strain produce biofilm was in tryptic soy broth (TSB) with 1 % (Glucose) cultured supplemented at 37 °C over night. For inoculum standar, were homogenized MRSA cells in solution saline (NaCl 0.85 %), the suspending was diluted to 0.5×10^8 CFU·mL−1 using O.D[11].

Antibiotic susceptibility test

Antimicrobial susceptibility test (AST) was depended according to (Wayne, PA: NCCLS, 2002)[12]. AST

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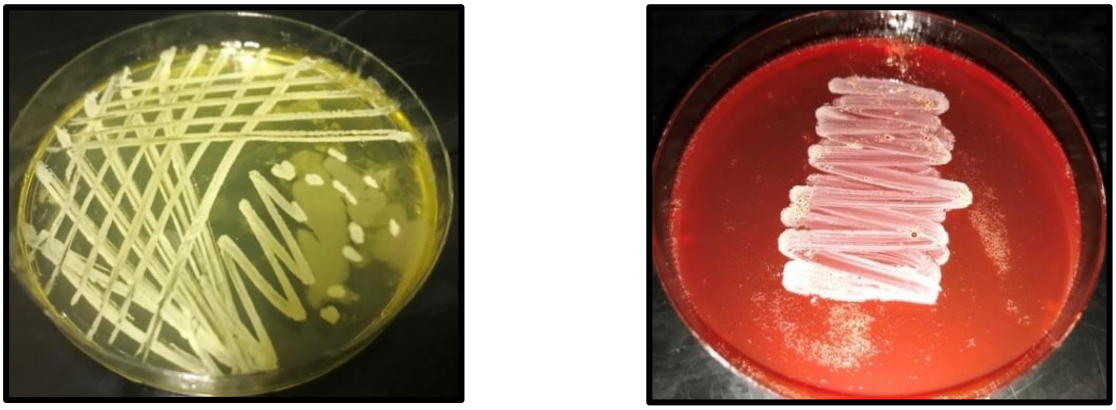

A B

Figure 1: *Staphylococcus aureus* (A) On mannitol salt agar, (B) on Blood agar at 37°C for 24 hrs.

Figure 2: Antibiotic susceptibility test of *Staphylococcus aureus.*

Table 1: Absorbance for MRSA biofilm at 540 nm*.

Isolate code	Absorbance ± SD
M1	0.601 ±0.079
M2	0.362 ±0.185
M3	0.742 ± 0.124
M4	0.480 ±0.160
M5	0.384 ±0.112
M6	0.554 ±0.166

including Amoxicillin (20µg/disc), Chloramphenicol (30 µg/disc) Erythromycin (15 µg/disc), Gentamicin (1µg/disc), Methicillin (5µg/disc)[13].

MRSAcin activity assay

The MRSAcin producer was done according to (Mais E Ahmed and Muna T Al Mossaw 2017)[14]. The antimicrobial activity of crude MRSAcin were examined for inhibitory activity against different strains MRSA causing biofilms by using agar well diffusion assay (AWD) assay. MRSAcin of measurement by serial two-fold dilutions of (crude MRSAcin) cell-free supernatant[15].

Plant material and extraction procedure

From a local herb store collection the black seeds crash as powdered (50 g) of seeds powders were extracted with (300 ml) hexane for (5 hours) and a half in soxhlet apparatus on a temperature of 70ºC. The extract was concentrated under reduced pressure in rotary evaporator to the 1/8 of it volume. Then tokens 25 ml of concentrated extract (which contain hexane) were distilled for 1 hr. The distillate was dried over magnesium sulphate[16]. Anti-Bacterial activity testing and the newly synthesized compounds were screened for their antibacterial activity against MRSA, using the micro dilution broth procedure[17].

Biofilm assay

Method described by (Singh R *et.al*., 2010)[18] Screening beast strain of MRSA biofilm formation by cultured in Brain Heart Infusion (BHI) broth incubated at 37ºC for 18 hrs was diluted in BHI broth after that bacterial culture with (MacFarland tube no. 0.5) compared were used to inoculate pre-sterilized 96-well polystyrene microtiter plates and later incubated for 24 hrs at 37ºC after incubation, all wells were washed with (PBS) for the detection of unattached cells, after ward, 25 µl of 1% crystal violet was added to each well, shaking the plates three times to help the colorant to get the bottom of the well. After 15 min at room temperature, each well was washed with (200 µl) sterile (PBS). The crystal violet bound to the biofilm was extracted later with 200µl of (ethyl alcohol), and then absorbance was determined at 540 nm in an ELISA reader (Beckman coulter, Austria). Controls were performed with crystal violet binding to the wells exposed only to the culture medium without bacteria. All the assays were performed in triplicates. Harley, J. and Prescott, H, method was performed to determine the viability of bacterial cells within the biofilm[19], all procedure describe above it repeat for detection activity of both nigella oil and MRSAcin against M7 strain. At concentration (0.25, 0.5, 0.75, 1.0) µg/mL for nigella oil and MRSAcin (50, 75, 100, 125)µg\ ml.

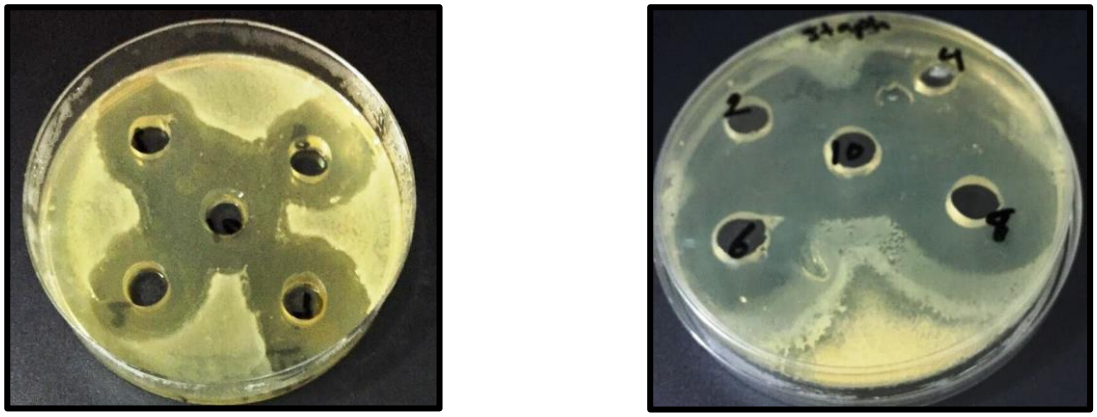

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Figure 3: MIC for (A) *Nigella* oil at (0.25, 0.5, 0.75, 1.0) µg/mL, (B) MRSAcin on (50, 100 125)µg\ ml. on Muller Hinton Agar at 37°C for 24 hr.

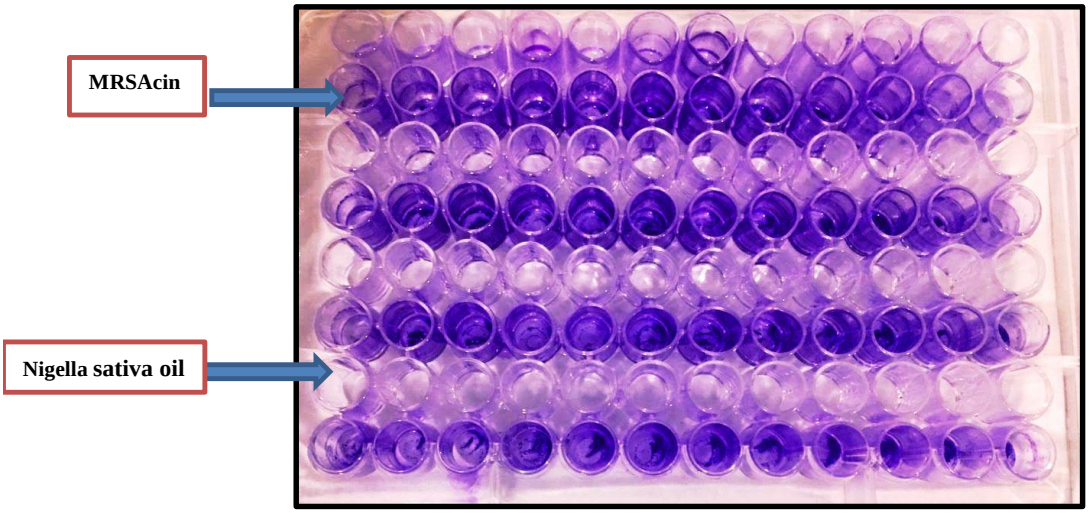

Figure 4: The comparative bactericidal effect among MRSAcin and Nigella sativa oilon MRSA strain.

Agar Well Diffusion Assay (AWD):

Efficiency purified and crude MRSAcin and *Nigella sativa* (seed oil) measurement activity was carried out by serial two-fold dilutions method by AWD[13].

RESULT AND DISCUSSION

Isolation and Identification

after culturing on Mannitol salt agar MRSA isolates have the ability to ferment mannitol and turn the color of medium from red to yellow were classified as a presumptive *S. aureus* isolates, thus this medium was considered as selective and a differentiated medium to *Staphylococcus spp*. On Blood agar β-hemolysisthe isolates showed this description is mentioned by (20) Figure (1).

Identification of MRSA

The Fifteen MRSA collected isolates were initially diagnosed *S. aureus* giving a positive result as MRSA by AST and Vitek 2 System detected as *S. aureus* on (BPA) appear gray to black colony surround with halo zone that conceder enrichment media for *S. aureus*

Antibiotic susceptibility test (AST) of Staphylococcus aureus

Figure (2) **s**howed various of susceptibilities to different antibiotics among isolates that were observed by **(**Disk diffusion method**)**. Out[15] *S. aureus* isolates were multi-resistance for antibiotics with a high level against Methicillin, Penicillin G, Cefoxitin, Erythromycin, Oxacillin, Chloramphenicol and Tetracyclin while sensitivity to Azitromycin this result was similar to that obtained by[21] on other hand agree with[22]. Sixty-four (30.62%) isolates were resistant to methicillin.

Fifteen MRSA isolates screening to detection were the thickest biofilm formation in Table (1) showed (6) strains adhesion formation. Obviously, MRSA (M3 strain) achieved the highest biofilm thickness.

The differences in biofilm thickness resulted from different reasons such as differences in isolates capacity to form biofilm furthermore, perhaps the primary number of cells that succeeded in adherence, and the differences of quantity and quality that produced from each isolate play an essential as well as important role[23].

Comparsim MRSAcin and Nigella oil

Well diffusion method was used to measure MRSA sensitivity to word MRSAcin MIC the result recorded below in figure (3), At concentration (50, 100**,** 125)µg\

ml while *Nigella* oil at (0.25, 0.5, 0.75, 1.0)µg/mL the inhibition zone diameter were (19, 17, 14 and 11) and (14, 11, 9 7) mm respectively.

Antimicrobial activity of *N. sativa* oil is attributed mainly to its phenolic constituents of the essential oil compartment thus, thymoquinone followed by its related compounds such as thymohydroquinone, dithymoquinone, and thymol along with carvacrol plays major role in antimicrobial activity[24,25]. The antibacterial activity of *N. sativa* oil against Gram-positive and Gram-negative bacteria were in agreement with other studies antibacterial assay methods used, percentage of active components in the oils.The present study acceptable with the previous study of Mais E Ahmed and Muna T Al Mossaw (2017) were investigated the crude MRSAcin effective agent wide range of microorganism involving Gram pathogeneses[14].

Biofilm formation

The antibiofilm activity of MRSAcin and *Nigella* oil at different concentration was carried out by using microtiter plate method, the results showed MIC of MRSAcin at (50 µgl/ml) more affected against MRSA biofilm then *Nigella* oil the at (0.25mg/ml) concentration against MRSA biofilm as showed in figure (4). Bacteriocins had the highest bactericidal activity against both planktonic cells and biofilm cells[25], and the anti-MRSA activity was reduced when *Nigella* (methanolic) extracts sativa seeds were used in combination with the (pain reliever and the fever reducer) (acetaminophen). The cell wall of Gram-positive bacteria is the target of anti-staphylococcal activity of *Nigella sativa* which responsible for changing the morphology of cell[27].

CONCLUSION

This study suggested the effects of bacteriocins (MRSAcin) against most isolate of MRSA. The tested bacteriocins showed the highest bactericidal activation acte MRSA biofilm material and suggest that bacterocin from MRSA attacks biofilm cells more effectively than *Nigella* oil using although it was widely used at (first-line therapy) for difference MRSA infections.

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