Accelerating Burn Wound Healing by Egg Yolk Oil Extracts Against Pseudomonas Aeruginosa Biofilm Infection

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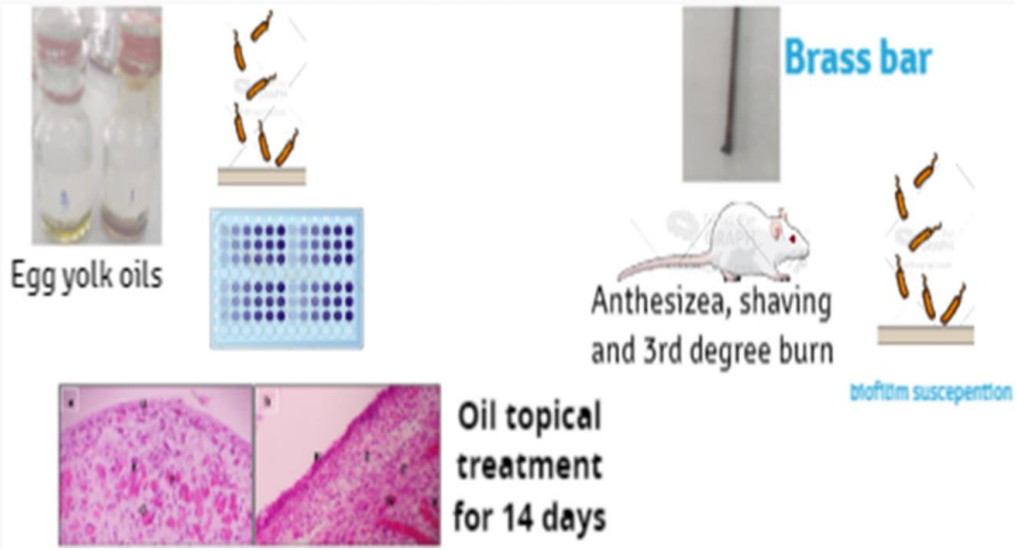
Abstracts

Biofilm can obstruct wound healing through a variety of mechanisms; the National Institutes of Health and the Centers for Disease Control and Prevention estimate biofilm is to blame for 65% to 80% of infectious diseases in humans. This study hypothesis was established through an experimental, laboratory-prospective study to assess burn wound healing if treated with egg yolk oil extracts against *Pseudomonas aeruginosa* biofilm infection, taking into account the importance of biofilm in postponing burn wound healing. Through using the microtiter plate method, the formation of biofilm was investigated in *P. aeruginosa* clinical isolates from patients associated with burn infections. Yolk oil extracts were evaluated in vitro for minimum inhibitory concentration (MIC) and antibiofilm inhibitory concentration against the strongest biofilm producer designated as (PA12) and (ATCC 27853) isolates. Our findings indicate that the MIC was determined at 45 mg/ml and 60 mg/ml respectively. In the biofilm inhibition assay, a sub-inhibitory concentration (SIC) of 30 mg/ml was screened, resulting in a remarkable statistically significant reduction in biofilm quantity. The (SIC) was evaluated in vivo against an established *P. aeruginosa* biofilm infection on a conventional Fischer F-344 rat burn wound model. Histological examination of wound healing in burned rats treated topically with yolk oil extracts revealed extensive re-epithelialization and anti-inflammatory benefits without tissue scarring.

Graphical abstract



Keywords: biofilm, burn wound, egg yolk oil extracts, *Pseudomonas aeruginosa*, topical treatment.

1. Introduction

Burn injuries are a leading cause of mortality and disability around the world. Burn injuries are still common worldwide despite a decline in high-income nations, with low- and middle-income regions accounting for 90% of all burns (Smolle et al., 2017, Greenhalgh, 2019) (Smolle et al., 2017). Worldwide, the World Health Organization (WHO) estimates that 11 million burn injuries of all kinds occur each year, 180,000 of which are fatal (WHO,2018), particularly due to drug-resistant bacterial infections, which frequently lead to lengthier hospital stays, slower wound healing, higher expenditures, and higher

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death (Vinaik et al., 2019).

Microorganisms can colonize the environment through the survival strategy of adhering to and growing as a biofilm on a surface. Bacteria regularly transition from a planktonic to a sessile phenotype: this phenotypic variation is important to the cell since it enables quick environmental adaptability (Grice and Segre, 2011, Lebeaux and Ghigo, 2012). The National Institutes of Health and the Center for Disease Control believe that biofilms are responsible for 65 to 80% of human illnesses. Bacterial biofilms have a multitude of tolerance mechanisms that make them extremely resistant to antimicrobial therapy. (Jamal et al., 2018, Ramos-Gallardo, 2016).

Multidrug resistance represents one of the most severe risks to global health. The "ESKAPE" bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) were first outlined in 2008 and proceeded to be the main cause of MDRO human infections worldwide (Sy et al., 2022). *P. aeruginosa* is a significant MDR pathogen, and one of the characteristics of the infection is its capacity to attach to and spread across medical equipment, including catheters, contact lenses, and wound dressings, by building powerful biofilms (Olivares et al., 2020). The development of efficient biofilm control strategies and therapeutic interventions highly depends on the identification of potent agents capable of sterilizing *P. aeruginosa* biofilms. Since domestic therapies for wound healing, infection prevention, scar tissue reduction, and availability remain a significant challenge (Guoqi et al., 2018, Gerner et al., 2020). The composition of the egg is dense in nutrients containing proteins and lipids with remarkable functional capabilities, as well as biological activity. During the past few years, lipids from egg yolks, especially egg yolk oil, phospholipids, and fatty acids that have the anti-inflammatory ability, antioxidant properties and cardiovascular protection, as well as memory-enhancing properties, have been extracted and investigated (Xiao et al., 2020, Rastegar et al., 2011).

It was discovered that various avian egg parts might be used to make cosmetic and therapeutic products. According to the literature, albumin or egg white was used to create a flexible bacteriostatic coagulum for the treatment of burns (Bourin et al., 2011, Rastegar et al., 2011). The development of novel medicines, the wise and proper use of antibiotics, and improved infection control techniques are critical in the ongoing war against multi-resistant pathogens. It has been complicated to consistently demonstrate the efficacy of various treatment modalities, such as topical dressings, in creating, maintaining, and controlling a moist wound healing environment (Nuutila and Eriksson, 2021). Since they are so important to burn research, experimental burn models are always required. The classic Fischer F-344 rat burn model was employed in this study to assess yolk oil's potential as a biofilm-controlling and anti-inflammatory therapy in vitro using the microtiter plate method, as well as in vivo as topical dressings by examining histological sections.

2. Materials and Methods

2.1 Characterization of the study

This experimental, lab-based study evaluated burn wound healing dressed with yolk oil extracts against *P. aeruginosa* biofilm infection in a rat burn model. Fourteen clinical isolates from burn patients were randomly selected and (ATCC-American Type Culture Collection) of *P. aeruginosa* (ATCC 27853) as a reference isolate as a positive biofilm producer strain was obtained from Medya Diagnostic Center (MDC). The strong biofilm producer isolate was selected and treated with yolk oil extracts to investigate the antibacterial and antibiofilm inhibitory concentrations.

2.2 Preparation of extracts:

2.2.1 Extraction of yolk oil

Fresh farm eggs were purchased, the eggshells were carefully cracked, and the egg yolk was kept separated from its white counterpart. A streamlined heating procedure was undergone to yield the brownish-orange egg yolk oil (Hu et al., 2012). The yolk oil has been extracted by two methods. The yolk oil has been extracted by two methods:

2.2.1.a Heat extraction method

Preferably, the egg yolk is heated to create the egg yolk oil or ointment. The heat treatment is most conveniently done in two steps. For at least five minutes, the initial heating stage is carried out at a temperature of roughly 100 °C. Boiling water can be used to perform the first stage, which helps to ensure that the ideal temperature is maintained throughout the process. When separating the egg yolk from the rest of the "hard-boiled" egg, it is preferred to keep it at a sufficient temperature for a long enough time to release its oil components. Next, prepare the heat-extracted yolk oil by gently heating the solidified egg yolk and taking care not to incinerate or char the extracted yolk oil (oil 1) (Hu et al., 2012, Mahmoudi et al., 2013).

2.2.1.b Solvent extraction method

The most widely accepted solvent for lipid extraction in food applications is hexane. Hexane will mainly extract simple triglycerides, which are neutral lipids. The boiling point of hexane is 69 degrees Celsius, making it an economical solvent for oil extraction. Therefore, the separated egg yolk was heated until it solidified and then extracted with hexane for one hour. The extracted oil was then separated and evaporated to yield solvent-extracted yolk oil (oil 2) (Wu et al., 2016, Mahmoudi et al., 2013).

2.3 Preparation and dilution of yolk oil

Both types of oils were dissolved with tween 80 preparing 30% v/v (3 ml oil + 7 ml tween) oil to get a

stock solution of 300 µg/ml. From this stock solution, four different final concentrations of 15, 30, 45, and 60 mg/ml were then obtained to assess the minimum inhibitory concentration and biofilm inhibition ability (Ahmed and Salih, 2019).

2.4 Biofilm formation assay:

The tissue culture plate method is the gold standard quantitative test for determining the formation of biofilm. trypticase soy broth 10 ml containing 1% glucose was inoculated with *P. aeruginosa* 1 x 10^6 CFU/mL and incubated for 24 hours at 37 degrees Celsius. The cultured *P. aeruginosa* was then diluted by a ratio of 1:100 with fresh media. Sterilized polystyrene wells of tissue culture plates (Sigma-Aldrich, Costar, USA) were filled with 200 µL of the diluted cultures. Similarly, ATCC isolate was incubated, diluted, and introduced to a tissue culture plate. A sterile broth of fresh medium was used as the negative control wells. The plates were incubated at 37°C for 24 hours. After incubation, the contents of each well were withdrawn by knocking them gently. To eliminate free-floating bacteria, four repetitions with 0.2 mL of phosphate buffer saline (pH 7.2) were used to rinse wells. Bacterial biofilms adhered to the wells were maintained with 2% sodium acetate and stained with 100 µL of 0.1% crystal violet for 15 minutes at room temperature. The surplus crystal violet was rinsed away with distilled water, and the plate was preserved for drying and measured by dissolving the stain in 95% ethyl alcohol. A Biorad micro-ELISA auto reader (model 680) was used to assess optical density (OD) at a wavelength of 570 nm (Christensen et al., 1985, Mathur et al., 2006). The experiment was conducted three times in triplicate. According to the criteria established by Mathur et al. (2006), biofilm formation was interpreted (Mathur et al., 2006, Ahmed and Salih, 2019).

2.5 Minimum inhibitory concentration assay

Microdilution assay is used to determine the minimum inhibitory concentration (MIC) in tissue culture 96-well plates following the National Committee for Clinical Laboratory Standards (CLSI, 2018) with modification; the antimicrobial effect of egg yolk oils was evaluated against the strongest biofilm producer *P. aeruginosa* (PA12) isolate. Cells of PA12 in the stationary phase equilibrated to OD550=0.5 were inoculated into 100 µl of Nutrient broth (NB; Oxoid) representing various concentrations (15, 30, 45, and 60mg/ml) of 30% v/v oil then aerobically incubated for 24 hours at 37 °C. The minimal inhibitory concentration (MIC) is the lowest concentration that inhibits growth. The subinhibitory concentration (SIC) was determined as the level below the minimum inhibitory concentration (MIC) and then screened to evaluate the anti-biofilm activity against the PA12 isolate. Three biological replicates for this assay were considered three times (de Rapper et al., 2016, Ahmed and Salih, 2019).

2.6 Biofilm inhibition assay

Microtiter plate assay was used to analyse the impact of egg yolk oils on biofilm formation. Briefly, overnight culture PA12 isolate and ATCC were subcultured in fresh NB broth (1 x 10^6 CFU/mL) in microtiter plates with flat bottom polystyrene (Costar/USA) containing and excluding SIC oils, and a positive control without yolk oil and a negative control without bacterial cells were prepared, then incubated at 37 °C under a static condition for 24 hours. Removal of liquid cultures from the well plates was carried out, and phosphate-buffered saline (PBS) was applied to wash wells three times before being stained with a 1% crystal violet solution. Afterwards, distilled water has been used to clean the wells, and the stain was quantified by dissolving it in ethyl alcohol (95%). An Elisa reader assessed the colourful suspension's optical density at 570 nm (Epson, Biotek, UK) and the wells were washed three times with phosphate buffered saline (PBS) and stained with a 1% crystal violet solution. The wells were then washed with distilled water and the stain was quantified by dissolving it in ethyl alcohol (95 %). The coloured suspension was measured at 570 nm optical density by an Elisa reader (Epson, Biotek, UK) (Ahmed and Salih, 2019).

2.7 Biofilm inhibition in vivo (rat burn model)

2.7.1 Experimental animals and design

Twenty healthy adult female albino rats (*Rattus norvegicus*) weighting (180-200 gm) from the animal house belonging to the Biology Department/College of the Science/University of Salahaddin were used in the present study which was designed to study the anti-biofilm and the anti-inflammatory effects *in vivo*. The rats were housed under laboratory standards. Under the supervision of the Animal Care Committee of the Veterinary Organization all experiments, care and the sacrifice procedure were executed. Randomly the animals were assigned into five groups (G): G1: Control rats without burns. Burned skin rats (BSR) + phosphate-buffered buffered saline (G2) G3: (BSR) + PA12 (1 x 10^6 CFU/mL). G4: (BSR) + PA12 + oil 1 (topically treated). G5: (BSR) + PA12 (1 x 10^6 CFU/mL) + oil 2 (topically treated).

2.7.2 Burn model and infection

The dorsal skin (1 cm^2) of the rats was shaved after anaesthesia by intramuscular injection of ketamine hydrochloride (90 mg/Kg) body weight. A standard 3rd-degree Fischer F-344 rat burn model has been established (Meyer and Silva, 1999) utilizing a brass bar which is a burning tool consisting of two parts, a handle that is terminated with a 1 cm^2 steel plate (Fig:1). The brass bar was heated in boiling water and using a thermometer, the brass bar's temperature was monitored as 100^o C. The bar was held in contact with the shaved animal skin for 20 seconds. The bacterial cells were cultured overnight at 37 °C

in Trypticase Soy Broth, and then 100 µl of *P. aeruginosa* suspension was applied with a sterile pipette tip to the burn region immediately post-burn. Control wounds were treated with 100 µl of sterile PBS (Brandenburg et al., 2019, Rastegar et al., 2011).

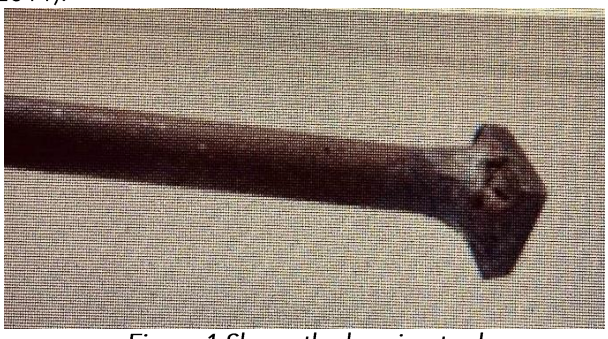

Figure:1 Shows the burning tool

2.7.3 Oil administration

Following the activation of a biofilm infection for 48 hours, the appropriate treatment was topically given to the treated groups for 14 days at a rate of 100 l per day to the burned area (Brandenburg et al., 2019).

2.7.4 Detection index

2.7.4.a Visual observation

The animals' wounds were photographed daily for signs of infection, erythema, swelling, lesion, or tightening of the skin around the burns, and then they were sacrificed with an anaesthetic overdose (Rastegar et al., 2011, Brandenburg et al., 2019).

2.7.4.b Quantitative bacteriology

On the 4th and 14th postoperative days, wounds exudates from treated groups were collected by sterile swabs for bacterial isolation, which were cultured on selective MacConkey agar plates (Yenilmez et al., 2015).

2.7.4.c Pathological skin sections

The burnt areas were excised and fixed in formalin. Prepared tissue slices were stained with hematoxylin and eosin (H and E). several histological scores for determining the severity of inflammation included the presence or absence of epithelial covering (such as crusting or spongiosis), granulation tissue and collagen matrix organization (such as oedema or hemorrhage, as well as a high density of eosinophilic collagen matrix), and inflammatory infiltrates (such as neutrophil anaphylaxis) (Galeano et al., 2006, Yenilmez et al., 2015, Brandenburg et al., 2019).

3. Statistical analysis:

The research findings were analyzed using the GraphPad Prism 8.0 software. One-way analysis of variance (ANOVA), t-test, and correlation was used for Multiple isolates comparison of *P. aeruginosa* in the biofilm-forming range relative to the reference strain. The results were displayed as mean ± standard error (SE). At P-value was <0.0001, which is statistically significant.

4. Results

4.1 Biofilm formation

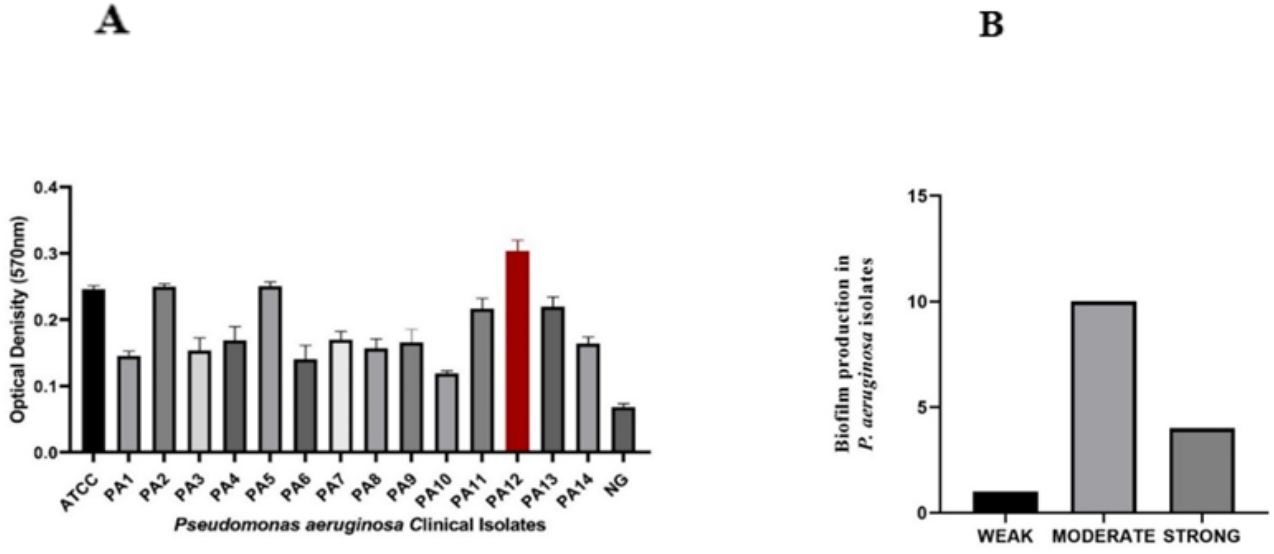

Figure: 2 (A) Biofilm formation among P. aeruginosa clinical isolates. (B) Distribution of biofilm formation among clinical isolates of P. aeruginosa.

4.2 Biofilm formation interpretation

Table 1: Interpretation of bacterial biofilm formation in *P. aeruginosa* isolates.		
Mean OD values	Adherence	Biofilm formation
<0.120	None	Weak
0.120 - 0.240	Moderately	Moderate
>0.240	Strongly	Strong

4.3 Minimum inhibitory concentration of yolk oils

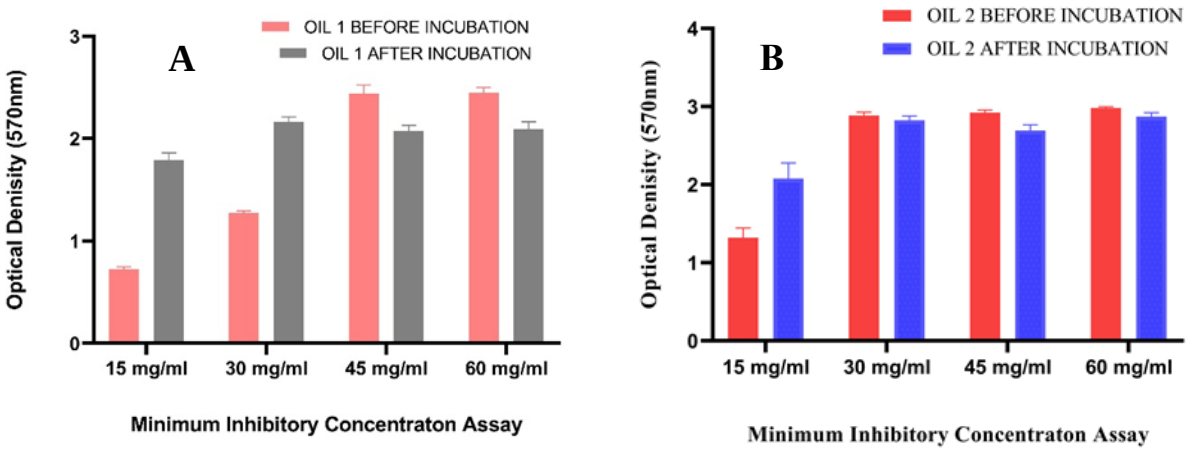

Fig. 3 (A) MIC values of heat extracted yolk oil (oil 1). (B) MIC values of solvent extracted yolk oil (oil 2).

4.4 Biofilm inhibition status

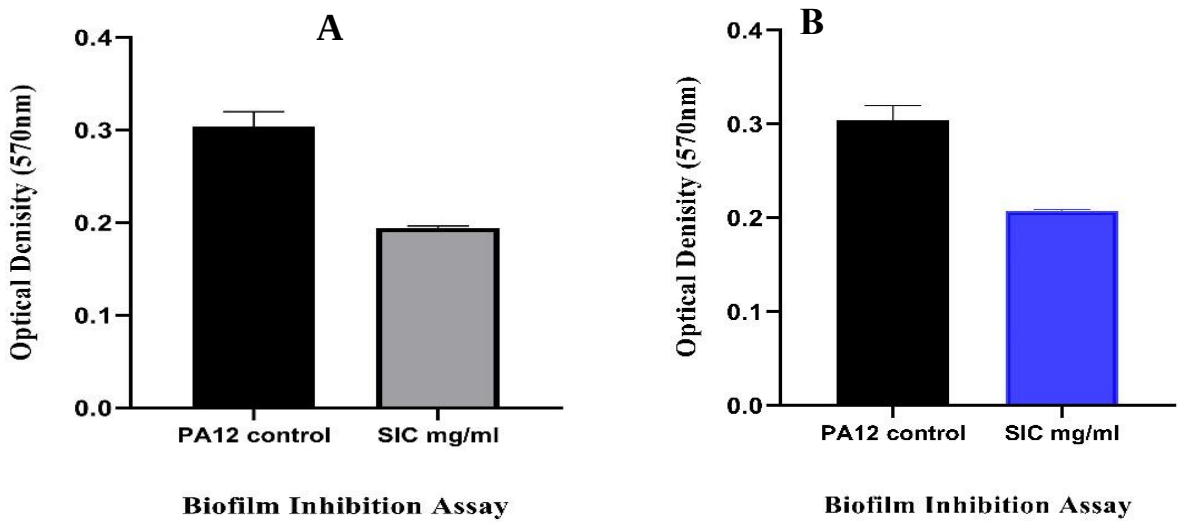

Fig. 4 Comparison of biofilm formation status. (A) Quantitative measurement of biofilm inhibition in PA12 treated with heat extracted yolk oil SIC 30 mg/ml. (B) Quantitative measurement of biofilm inhibition in PA12 treated with solvent-extracted yolk oil SIC 30 mg/ml.

4.5 Biofilm inhibition status

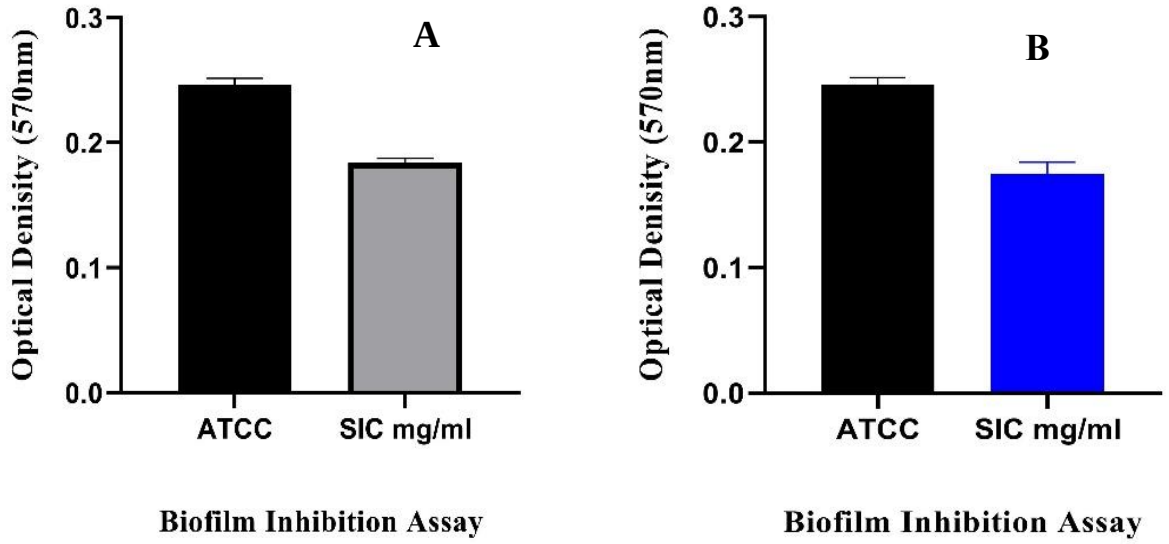

Fig. 5 Comparison of biofilm formation status. (A) Quantitative measurement of biofilm inhibition in ATCC treated with heat extracted yolk oil SIC 30 mg/ml. (B) Quantitative measurement of biofilm inhibition in ATCC treated with solvent-extracted yolk oil SIC 30 mg/ml.

4.6 Gross images of 3rd degree burn wounds

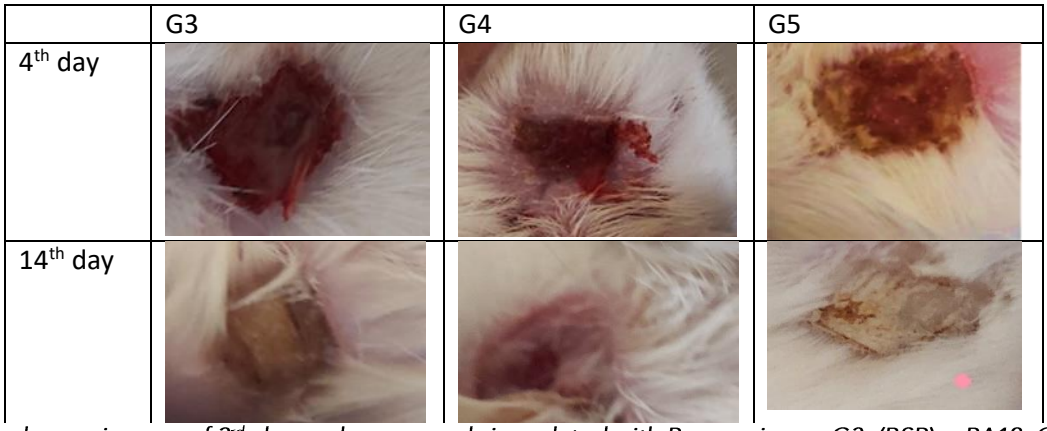

Fig 6: Typical gross images of 3rd-degree burn wounds inoculated with P. aeruginosa, G3: (BSR) + PA12. G4: (BSR) + PA12 + oil 1 (topically treated). G5: (BSR) + PA12 + oil 2 (topically treated).

4.7 Skin sections of untreated and treated 3rd degree burn wounds

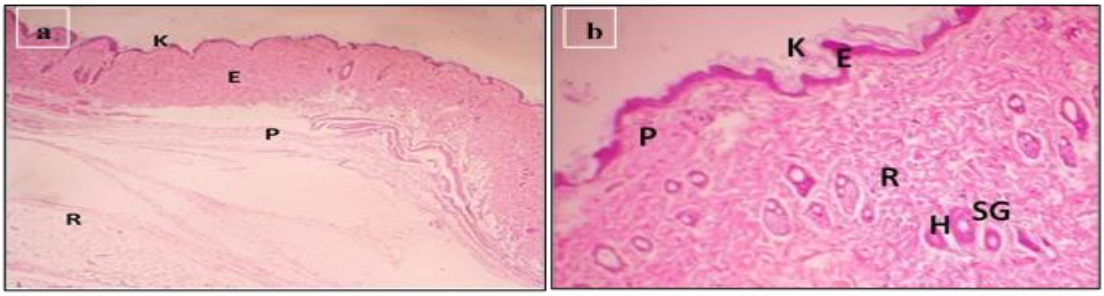

Fig. 7 a and b. The skin section of the control group [G.1] shows a normal epidermis formed of the keratinized stratified squamous epithelium [E] and dermis. The dermis consists of a superficial papillary layer [P] and a deeper reticular one [R]. b. showed sebaceous glands [Sg] and hair follicles [H] are seen in the mid to upper dermis and some were seen near the hypodermis. [Group. 1: H and E; a. x100; b. x400].

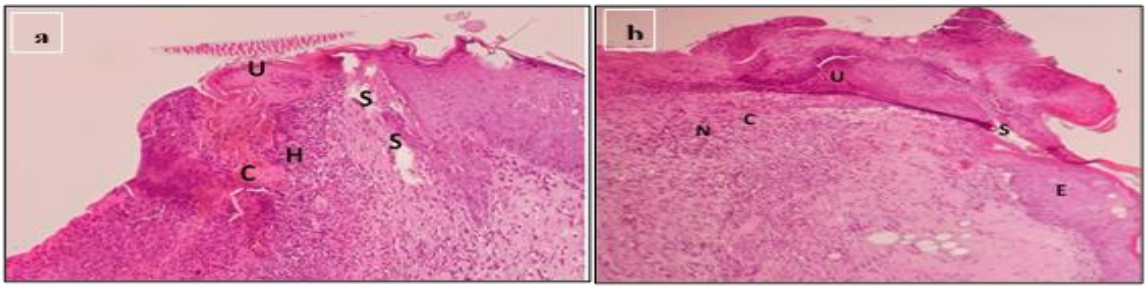

Fig. 8 section of burned skin of [G.2] a and b showing no epidermis (U), wide spacing of burn edges[S]. Notice: The damaged hair follicles [H] with lost architecture and loss of collagen fibers distinction (C). b. Showed loss of basement membrane and primary infiltration of macrophages. [Group. 2: H and E; a. x100; b. x400].

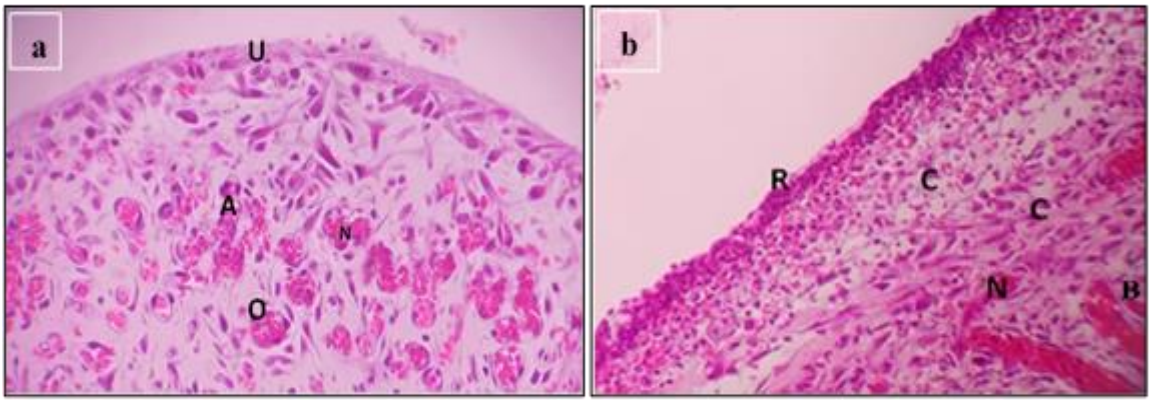

Fig. 9 sections of burned skin of [G.3] and [G.4]. a. represented [G.3] showing with no epidermis (U). Highly infiltration of inflammatory cells in the dermis region in addition to the loss of architecture with loss of collagen fibers distinction (collagenosis) (C) and highly infiltration of an inflammatory cell (acute (A) and chronic (O) inflammation) in the dermis region. b. represented [G.4] showing the first stage of repairing. Primary resqumation (R) Ulcer also appears to moderate inflammation (N). Blood congestion in a vessel (B) and collagenosis (C) [Group. 3 and 4: H and E; x400].

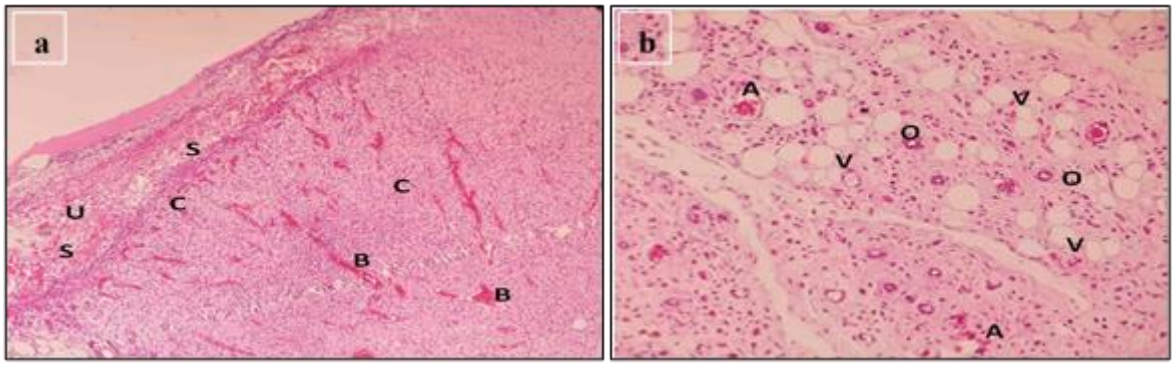

Fig. 10 section of burned treated skin of [G.5]. a. showing high rate of ulceration (no epithelial) (U), space in burn area moderate inflammation (N). Blood congestion in a vessel (B) and few rate collagenosis (C). b. exhibits numerous blood vessels inflammation high rate (acute (A) and chronic (O)) [Group. 5: H and E; a. x40; b. x400].

5. Discussion

Bacterial biofilm data calculation for individual isolates of *Pseudomonas aeruginosa* based on OD values obtained in (Fig.2) shows that most isolates were biofilm producers, which backs up previous previous studies (Ahmed and Salih, 2019, MosaChristas et al., 2021). The majority of isolates exhibited a biofilm-positive phenotype under the optimum TCP technique settings. In Table 1, four (6,66 %) of the isolates were categorized as strong biofilm producers, ten (66.66 %) as moderate, and one (26,66 %) as weak or lacking biofilm. Notable inhibitory activity in (Fig.3) was indicated in the yolk oil extracts of 30% used in four concentrations (15, 35, 45 and 60 mg/mL). Oil extracts have shown an effective inhibition and elimination capability against *P. aeruginosa* (PA12) at values of 45 mg/ml and 60 mg/ml, respectively.

Subinhibitory concentration (SIC) 30 mg/ml was screened against PA12 and ATCC 27853, which resulted in biofilm inhibition quantity which was statistically significant according to Fig. 4 and 5, all results were displayed as means ± SE. Both oils' significant impacts on PA12 and ATCC isolates provide optimism for patients with compromised immune systems, such as those with wound infection or cystic fibrosis, who face serious complications from *P. aeruginosa* biofilms. The occurrence of biofilms makes it increasingly challenging to get rid of the bacteria, which leads to the development of chronic infections (Lee and Yoon, 2017, Karami et al., 2019). Patients who are infected with *P. aeruginosa* biofilm may be more resistant to antimicrobial treatment than those who are infected with planktonic bacteria. Models for biofilm formation have undergone numerous iterations as a result of advances in biofilm research methods. In addition to genetic variables, sophisticated experimental approaches have revealed that biofilm growth consists of three distinct stages: initial attachment, maturation, and detachment of the biofilm. Among the biofilm's most important regulatory systems is the quorum sensing (QS) system (Lee and Yoon, 2017). Yolk oil extracts show a remarkable decrease in the expression of *P. aeruginosa* biofilm quantity which may be related to its impact on genetic or environmental variables, or maybe both. Our findings were confirmed by additional clinical studies demonstrating the effectiveness of yolk oil extracts application as antibiofilm therapy (Rastegar et al., 2011, Yenilmez et al., 2015, Guoqi et al., 2018).

The gathered laboratory results promote yolk oil extracts in the rat burn model to highlight the accelerated healing of infected 3rd-degree burn wounds with *P. aeruginosa* biofilm (Brandenburg et al., 2019). The results show a significant difference between groups in the reduction of infection according to visual observation images which reveal significantly more wound erythema and ulceration contrasted to treated groups (Fig. 6).

The quantitative bacteriology observation including microscopy and cultures, 4th-day results revealed that gram-positive cocci were ++, gram-negative bacilli were ++, staphylococci and *P. aeruginosa* respectively indicating a true infection were established. However, on the last day experiment greatly reduced in number and type of bacteria obtained in this aspect.

Topical treatment with the heating extracted yolk oil (oil 1), was most efficient in reducing and removing *P. aeruginosa* biofilms than solvent-extracted yolk oil (oil 2) and both shows increasing the healing, proliferation, differentiation, formation of new blood vessels, formation of a new epithelial cell layer in burn wound as our histological sections figures shows (Wu et al., 2016, Dhar and Han, 2020). In the "set proved recipe" of the Northern Zhou Dynasty, egg yolk oil was used for the first time as a topical treatment, and there are records of egg yolk oil being used orally to treat sores, fistulas, dysentery, and other diseases. This is supported by ancient monographs such as "Rihuazi Bencao," "Compendium of Materia Medica," "Chinese Eaglewood," and "Bencao Pinhui Jingyao."(Cansell, 2007, Bandelin and Tuschhoff, 1953). According to the "Chinese herbal medicine" publication, burns and severe burns, otitis media, sun-damaged-damaged skin, trauma, and parasitic lesions are just a few of the conditions that can benefit from the use of yolk oil (Zhao et al., 2011).

In vivo biofilm inhibition and wound healing rate was obtained through light microscope examination of skin sections of albino rats; consequently, the five

groups were as follows: Control albino rats' skin sections [G.1] showed the normal structure of the thin skin, it appeared formed of the thin epidermis and the thick dermis. The epidermis is made up of keratinocyte-rich stratified squamous epithelium that rests on a wavy basement membrane. The epidermis epithelial layers separate from the underlining dermis papillary by the basement membrane. The thick dermis region consists of both papillary and reticular regions. (Fig. 7). The dermis also contained many hair follicles with its associated sebaceous gland (Fig. 7 b).

Light microscopic examination of burned skin sections in albino rats [G.2] showed ulceration or no epidermis and the burned tissue edges separated from the unburned epidermis and dead tissue was found. The completely damaged hair follicles appeared [Figures 8]. The dermis showed collagenosis or loss of collagen fibers distinction, blood vessels were also detected (Fig. 8b).

Burned skin sections of albino rats inoculated with *P. aeruginosa* [G.3] showed that the normal structure of the skin was lost, epidermal disappear and a slight scab was formed that covered the burned area. The dermis region was invaded by many macrophage cells causing acute and chronic inflammation in the whole dermis region in addition to collagenosis (Fig. 9 a).

Burned skin treated with oil 1 [G.4] showed significant confirmatory evidence of the growth of the epidermis over the wound [re epithelialization or resquamation] but the different epidermis stratum layers cannot be distinguished. The underlying granulation tissue showed moderate inflammation with many congested blood vessels. Slightly reorganization in the reticular layer also can be observed (Fig. 9 b).

This group [G.4] exhibits a favorable recovery process of the wound, a positive effect on the reduction of burned area, and ultimately prevention of infection and accelerating burn wound healing compared with the oil 2 treated group. The aim of burn wound therapy is the enhancement of burn closing and resequmation of slugged skin function with good corrective results which is agreed with other previous studies (Rastegar et al., 2011, Brandenburg et al., 2019).

Oil 2 treated group [G.5] showed despite the presence of a high rate of ulceration (no epithelial) primary repairing steps can be detected. The dermis region occupied many blood vessels (congested and empty). Moderately invaded by inflammatory cells and the most inflammation were reach the chronic stage in addition to few rate collagenosis (C) (Fig. 10 a and b). In modern pharmacological studies, egg yolk oil has been found to have analgesic and wound healing, anti-ageing, memory improvement, blood pressure lowering properties as well as combination of antioxidant and anti-inflammatory properties with low cytotoxicity (Yenilmez et al., 2015).

The foregoing explanations imply that a topical treatment using egg yolk oil could be an excellent solution to minimize burn wound injury, particularly in biofilm-infected burn wounds.

6. Conclusions

Based on the study's findings, *Pseudomonas aeruginosa* biofilm formation is inhibited and reduced in vitro by yolk oil extracts.

In comparison to the non-treated groups, accelerated burn wound healing in treated groups was realized through plentiful re-epithelialization without scarring.

A combination of yolk oils treatment with antibiotics will minimize infection which helps in the management and mitigation of biofilm-infected burn wounds.

Yolk oil's precise role in the process of healing is a mystery. Furthermore, additional research investigating the impact of various yolk oil compartments is recommended.

7. Acknowledgements

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