EVALUATION OF A TOPICAL FORMULATION CONTAINING EXTRACTS OF TURMERIC, POMEGRANATE AND BANANA PEEL ON *STAPHYLOCOCCUS AUREUS* PATHOGENESIS AND STUDYING ITS WOUND HEALING PROPERTIES.

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Abstract: *Staphylococcus aureuscauses a range of infections, ranging from a simple boil to antibiotic-resistant infections to flesh-eating infections.Increased resistance to conventional antimicrobials has complicated the treatment of wound infections caused by Staphylococcus aureus. Phytochemicals, which are naturally present in plants have tremendous therapeutic potential and can be exploited in overcoming these infections. In this study, peels of pomegranate and banana, an agro-industrial waste were tested for their therapeutic properties to investigate their effectiveness as a new source of antimicrobials and wound healing agents. Powdered peel extracts of pomegranate and banana along with turmeric were prepared to study their antimicrobial activity along with their effect on Staphylococcus aureus pathogenesis. MIC/MBC values were determined and the presence of important phytochemicals were analyzed. A topical gel was formulated and evaluated for its physicochemical characteristics, stability and wound healing properties (Angiogenesis). According to the achieved results, banana peel extract showed the highest antimicrobial activity as compared to the other two. The virulence factors like Proteinase and Phospholipase of S. aureus were inhibited by the tested extracts.Therapeutically important phytochemicals such as tannins, flavonoids, glycosides, terpenoids and saponins were present in the studied extracts. The formulated gel showed enhanced angiogenesis, indicating its role in wound healing as evaluated by using chorioallantoic membrane assay.*

Key words: *Antimicrobial resistance, banana peel, pomegranate peel, Staphylococcus aureus, topical gel, turmeric, wound healing.*

I. INTRODUCTION:

Skin and wound infections have afflicted humanity since time immemorial. Nearly everyone has already suffered an open skin wound as a result of a cut, scrapes or scratches, burns, diseases (e.g., diabetes) or surgical interventions. On some occasions, those wounds can easily be contaminated by different pathogens found in the surrounding environment, endogenous microbes living in the mucous membranes, or by the microflora available on the adjacent skin. Frequent wound colonizers include *Staphylococcus aureus*, *Pseudomonasaeruginosa*, *Escherichia coli*, *Candida albicans* and streptococci either in their free-floating state or in biofilms where they are attached to a surface and protected by surrounding extracellular matrix produced by them (1). Several factors determine transition from colonization to infection: Weakened/suppressed immune system of the host, Diabetes, Malnutrition, the bio-burden itself and the virulence of the organisms.

Staphlococcusaureus is the causative agent of a considerable proportion of skin and wound infections. It is both a commensal bacterium and a human pathogen capable of causing superficial skin infections such as impetigo and infected abrasions as well as more complicated skin infections such as cellulitis, folliculitis, subcutaneous abscesses, and infected ulcers and wounds. These *S. aureus* skin infections constitute a serious threat to public health given the massive numbers of infections as well as the widespread emergence of antibiotic resistant strains such as methicillin-resistant *S. aureus* (MRSA), including hospital and community-acquired MRSA (CA-MRSA) infections (2), (3) & (4).

Skin and wound infections interfere with the normal healing process and can create additional tissue damage. Despite the numerous existing antimicrobial agents available today, topical skin infections commonly occur and often present therapeutic challenges to practitioners. There is a dire need to explore new antimicrobial agents because of the emergence of antibiotic resistant strains and adverse effects of synthetic drugs on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions. This has revived the search for alternative natural drugs that would be safer than conventional drugs, besides being economical, effective and easily available.

The use of plants for treating diseases is as old as the human civilization. Phytochemicals, which are naturally present in plants have tremendous therapeutic potential which can be exploited in curing various ailments Plants are more potent healers because they promote the repair mechanisms in the natural way. Turmeric, an indigenous herb in Southern Asia is well recognized with antioxidant and anti-inflammatory properties. While herbals and spices have been in the scientific limelight and investigated for their antimicrobial properties for centuries, recently much attention has been shifted to peels of fruits and vegetables. From different studies conducted on peels, it has been reported that peels of fruits and vegetables, considered as an agro-industrial waste, hold a tremendous potential to serve as a source of newer, effective, safer and better antioxidant and antimicrobial agents.They could be used as true antibiotics as they are available for no cost, have no side effects and the most important benefit is that antibiotic resistant pathogens will be easily killed by these new and natural antimicrobials because they will take at least a few decades to get mutated and resistant to them.

In the present study, a topical formulation of extracts of pomegranate and banana peel along with turmeric was used to study its effectiveness against*Staphylococcus aureus* skin and wound infections.

II. MATERIALS AND METHODS:

MATERIALS:

Pomegranates, bananas and turmeric were procured from the local market of Mumbai, Maharashtra. All chemicals were of pharmaceutical grade.

METHODS:

1. PREPARATION OF EXTRACT:

Pomegranate and banana peels were separated and washed with tap water and subjected to drying in a microwave uptodryness. The dried peels were ground to coarse powder. To prepare samples: 20 g of ground peel and turmeric powder were separately soaked in 100 ml of methanol. The samples were incubated at 37°C for 24h in a shaking incubator with 200 rpm. After this, the samples were filtered with Whatman no. 1 filter paper and the residue was re-extracted further following the same procedure. Extracts were air-dried under a stream of air and then dissolved in methanol.

2. DETERMINATION OF ANTIMICROBIAL ACTIVITY BY AGAR WELL DIFFUSION ASSAY:

Culture suspensions of each of 24h old *Escherichia coli* and *Staphylococcus aureus* having 0.1 O.D was prepared. Two Nutrient Agar plates were prepared by bulk seeding two NA butts with 0.1ml of culture suspension of *Escherichia coli* and *Staphylococcus aureus*. Four wells were created onto each agar plates using a St. cork borer. 100µl of methanol extract of powdered peel of pomegranate, banana and turmeric was added into the prepared wells. Methanol was used as control. Plates were incubated at 37°C for 24hrs. The zone of inhibition surrounding the wells were observed and measured in mm.

3. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC):

The MIC was determined using the serial two-fold dilution method. 0.1 ml of culture suspension of *S. aureus* was added to all tubes and mixed well. The tubes were incubated at 37°C for 24hrs. Positive and negative controls were kept. The inhibition of growth at the minimum concentration was observed.

4. DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC):

The aliquot from MIC was streaked onto the surfaces of NA agar plates. Plates were then incubated for 24-48 h and checked for the presence of growth. The minimum bactericidal concentration (MBC) was defined as the minimum extract concentration that killed 99% of bacteria in the initial inoculums.

5. PHYTOCHEMICAL ANALYSIS:

PHENOLS:

Ferric chloride test: Extracts were treated with 3-4 drops of Ferric chloride solution. Development of bluish-black color indicated positive result.

FLAVONOIDS:

Alkaline reagent test: Extracts were treated with Sodium hydroxide solution, which showed increase in the intensity of yellow color which on addition of few drops of dilute Hydrochloric acid became colorless indicating the presence of flavonoids.

TANNINS:

5ml of extract was boiled in 10 ml of water in test tubes and then filtered. Then, few drops of 0.1% Ferric chloride was added. Formation of brownish-green color indicated a positive result.

TERPENOIDS:

2 ml of Chloroform and 3 ml of concentrated Sulfuric acid was added to 5 ml of extract. Formation of monolayer of reddish-brown color at interface indicated a positive result.

GLYCOSIDES:

Keller Killiani test: Extracts were treated with few drops of Glacial Acetic acid and Ferric chloride solution and then concentrated Sulfuric acid was added. A positive test showed the formation of two layers: lower reddish-brown layer and upper bluish-green acetic acid layer.

SAPONINS:

Froth test: To 0.5 ml of extract, 2 ml water was added and shaken. Formation of froth indicated a positive test.

6. VIRULENCE TESTS FOR *Staphlococcusaureus*:

A. PHOSPHOLIPASE ACTIVITY:

To 0.5 ml of extract, 0.1ml of *S. aureus* suspension was added. On St. Baird Parker's medium, this prepared mixture was spot inoculated at 30, 60, 90 and 180 minutes. *S. aureus* suspension was used as a control. The plates were incubated at 37°C for 24hrs. The zone of precipitation was observed.

B. PROTEINASE INHIBITION ASSAY:

To 0.5 ml of extract, 0.1ml of *S. aureus* suspension was added. On St. Skim milk agar plate, the prepared mixture was spot inoculated at 30, 60, 90 and 180 minutes. *S. aureus* suspension was used as a control. The plates were incubated at 37°C for 24hrs. The zone of precipitation was observed.

7. FORMULATION OF THE TOPICAL GEL:

The ointment was prepared by mixing warm petroleum jelly and methanol extracts of turmeric, pomegranate and banana peel at concentration of 15% (w/w).

8. EVALUATION OF THE PREPARED GEL:

COLOUR AND ODOUR:

Physical parameters like color and odor were examined by visual examination.

CONSISTENCY:

Smooth and no greediness were observed.

NON IRRITANCY TEST:

Prepared formulation was applied to the skin and observed for the effect.

DRY TEST:

Prepared formulation was applied on skin and its drying time and formation of film was observed.

STABILITY TEST:

Physical stability test of the prepared ointment was carried out for two weeks at various temperature conditions like 4°C, 25°C and 37°C.

9. EVALUATION OF THE WOUND HEALING PROPERTY (ANGIOGENESIS) OF THE PREPARED GEL BY USING CHORIOALLANTOIC MEMBRANE (CAM) ASSAY.

Fertilized chicken eggs were carefully surface sterilized with 70% alcohol and incubated at 37°C with 80% humidity. The eggs were inoculated with 10µl of respective standard or test sample and sealed and incubated in a vertical position (air sac upward). After 12 days, the eggs werebroken gently from the sides

of air sac and the inner shell membrane were removed carefully using forceps. The CAM was dispensed out on a Petri Plate containing 2ml normal saline, and the blood vessels were viewed and photographed. Erythropoietin was used as a positive control and petroleum benzene as a vehicle control.

III. RESULTS:

1. DETERMINATION OF ANTIMICROBIAL ACTIVITY OF THE THREE EXTRACTS BY AGAR WELL DIFFUSION ASSAY:

The antimicrobial activity of the extracts of peels of pomegranate, banana and turmeric were studied against two pathogenic bacterial strains, Gram positive: *Staphylococcus aureus* and Gram negative: *Escherichia coli*. Methanol was used as a control. Antibacterial potential of extracts were assessed in terms of zones of inhibition displayed in Table 1. Zones of inhibition were greater for *Staphylococcus aureus*as compared to *Escherichia coli*. Thus,*S.aureus*will be used for further studies. (Figure 3.1).

Figure 3.1 (a)

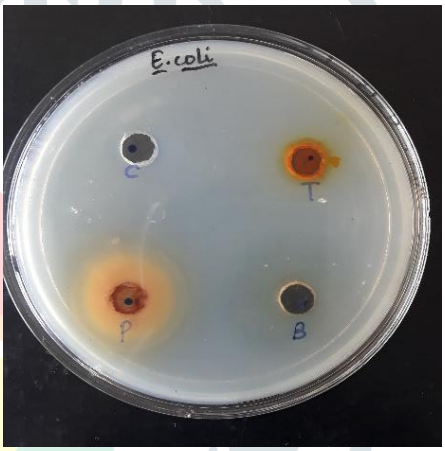


Figure 3.1 (b)

Zone of inhibition of the extracts against *S. aureus*Zone of inhibition of the extracts against *E. coli*

Key: P: Pomegranate peel extract

B: Banana peel extract

T: Turmeric extract

C: Methanol

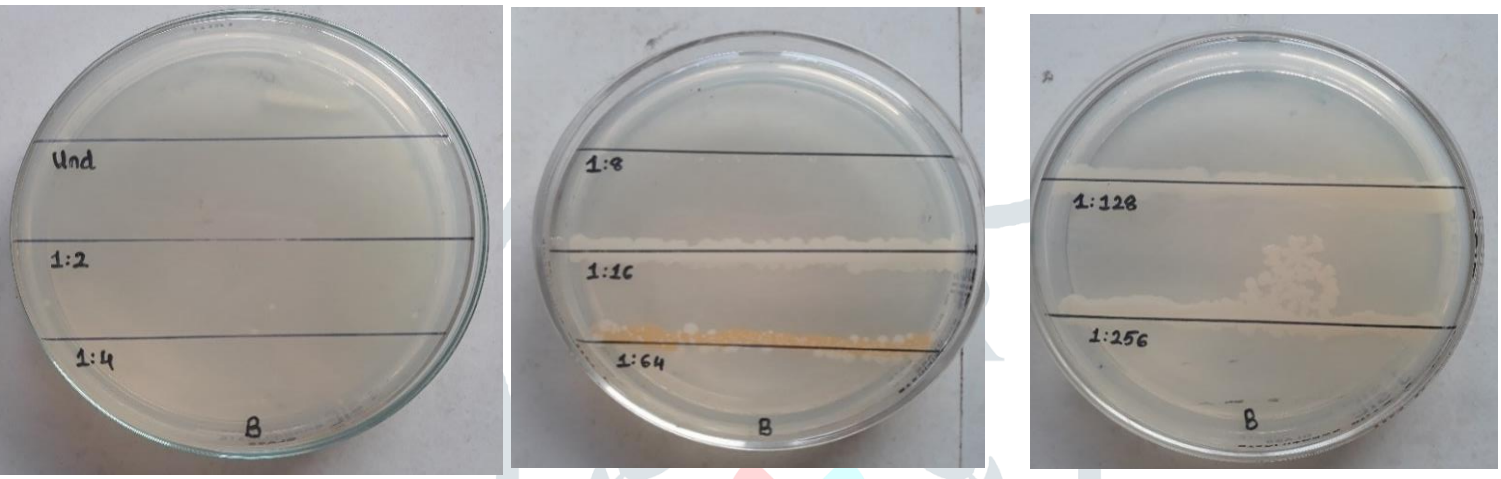
Bacteria	Zone of Inhibition (in mm)		
	P	B	T
S. aureus	27	28	13
E. coli	25	25	13

Table 1

Zone diameter of peel extract of pomegranate, banana and turmeric against *S. aureus* and *E.coli*.

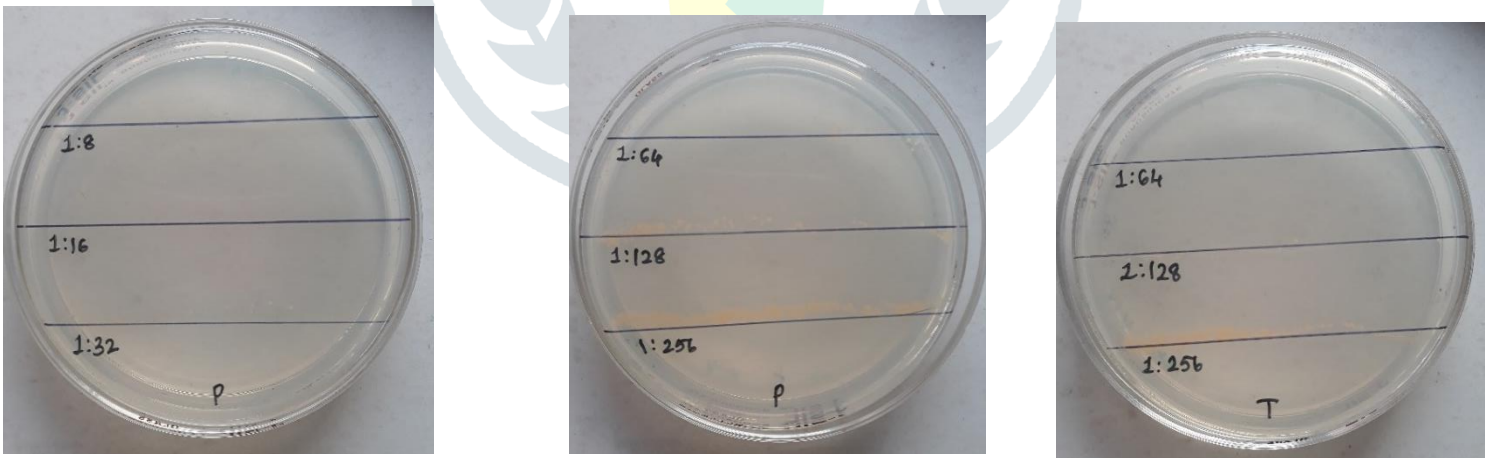
2. MIC/MBC:

MBC values of turmeric extracts were in the range of 1:64, followed by pomegranate peel in the range of 1:16 and the most potent inhibition showed by banana peel in the range of 1:4. The MBC values of the tested extracts against *S. aureus* were found to be same as the MIC. (Figure 3.2).



(a) (b) (c)

Figure 3.2 (a), (b) and (c): MBC results of banana peel extracts against *S.aureus*.



(d) (e) (f)

Figure 3.2 (d) and (e): MBC results of pomegranate peel extracts and (f) turmeric extracts against *S. aureus*.

3. PHYTOCHEMICAL ANALYSIS:

The different phytochemical constituents present in peels of pomegranate, banana; and turmeric is shown in Table 2. Banana peel showed presence of important tested phytochemicals whereas pomegranate peel lacked terpenoids, while flavonoids and saponins were absent in turmeric.

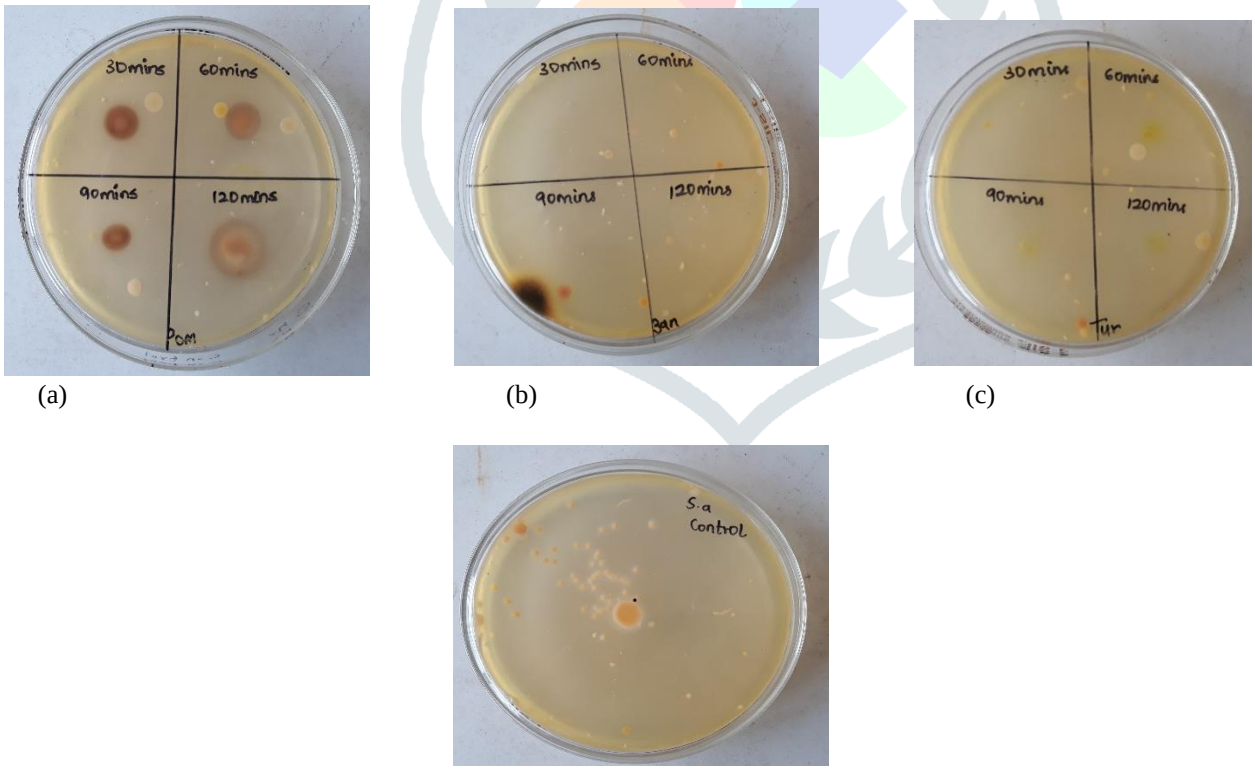
Test	P	B	T
1. Phenols a) Ferric Chloride test	+	+	+
2. Flavonoids a) Alkaline reagent test	+	+	-
3. Tannins	+	+	+
4. Terpenoids	-	+	+
5. Glycosides a) Keller Killiani test	+	+	+
6. Saponins a) Froth test	+	+	-

Table 2: Phytochemical analysis of peel of pomegranate, banana and turmeric.

4. VIRULENCE TESTS FOR *S. AUREUS*:

PHOSPHOLIPASE AND PROTEINASE INHIBITION ASSAY:

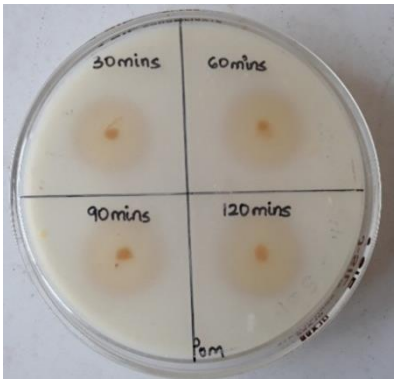
Presence of zone of inhibition was observed onlyfor pomegranate peel extracts. Extracts of banana peel and turmericshowed no clearance indicating that it has completely inhibited the virulent enzymes. (Figure 3.4).



(a) (b) (c)

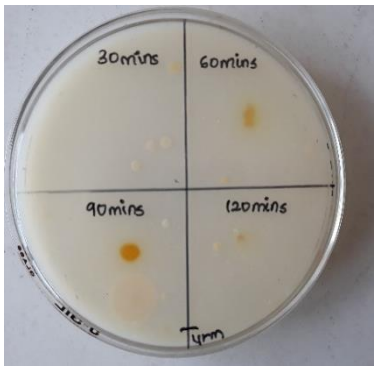
(d)

Figure 3.4 (a), (b) and (c) shows the phospholipase activity of pomegranate peel, banana peel and turmeric against *S.aureus* respectively. (d) *S.aureus* used as a control.

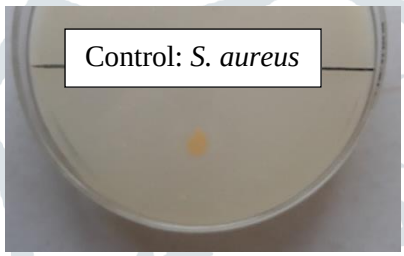


(e)

(f)



(g)



(h)

Figure 3.4 (e), (f) and (g) shows the proteinase inhibition activity of pomegranate peel, banana peel and turmeric against *S. aureus* respectively. (h) *S.aureus* used as a control.

5. EVALUATION OF THE PREPARED GEL:

The physicochemical properties were studied which showed satisfactory results and it was found stable at 4°C, 25°C and 37°C within the period of two weeks.

Physicochemical parameters	Observation
Colour	Brown
Odour	Characteristic
Consistency	Smooth
Non-irritancy test	Non-irritant
Dry test	Dry within 5 minutes.
Stability test	Stable

Table 3: Physicochemical evaluation of formulated gel.

6. CHORIOALLANTOIC MEMBRANE (CAM) ASSAY:

Angiogenic activity was determined using CAM Assay where erythropoietin was used as positive control, petroleum ether as vehicle control and uninoculated eggs as standard control. The mixture of three extracts was used as test. Erythropoietin which is the positive control (PC) was seen to enhance angiogenesis with increased branch point compared to the vehicle control (VC). The extracts showed enhanced angiogenesis indicating its role in wound healing. (Figure 3.6).

Fig. 3.6 (a) Uninoculated

Fig. 3.6 (b) Vehicle Control

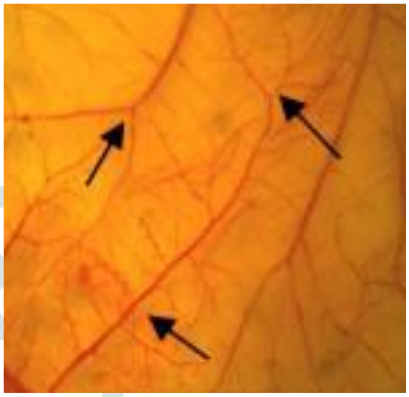


Fig. 3.6 (c) Positive Control

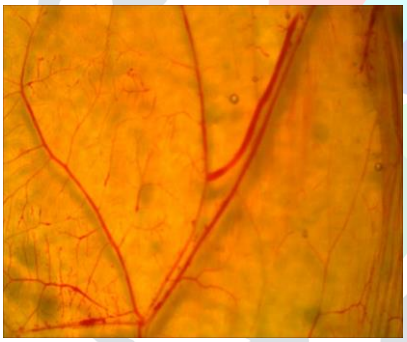


Fig. 3.6 (d) Test

IV. DISCUSSION:

*Staphylococcus aureus*is an opportunistic pathogen causing a plethora of commonly known infections ranging from superficial skin lesions to invasive subcutaneous abscesses and wound infections. With the increase of antimicrobial resistance becoming a challenge in therapeutic treatment, new and novel antimicrobials and wound healing agents are explored. In the present study, extracts of peels of pomegranate, banana and turmeric were used to exploit their effectiveness against the pathogenicity of *S.aureus*. Therapeutically important phytochemicals such as glycosides, terpenoids, saponins, flavonoids were found to be present in the three extracts.The antimicrobial activity of the powdered peel extracts was determined by using agar well diffusion assay displaying more potent effect against *S.aureus*. The MIC values were equal to the MBC values for the tested extracts suggesting a strong bactericidal action. The pathogenicity of *S.aureus* is attributed to its virulence factors that promote colonization of host tissues, invasions that promote bacterial spread in tissues and various other biochemical factors that enhance their survival. The virulence factors like Proteinase and Phospholipase of *S. aureus*were inhibited by the tested extracts. The formulated gel comprising of the three extracts showed satisfactory physicochemical

characteristics and stability at different temperatures within the two week period. The extracts showed enhanced angiogenesis indicating wound healing property as evaluated by chorioallantoic membrane assay.

V. CONCLUSION:

The prepared gel containing extracts of pomegranate peel, banana peel and turmeric was found to have antimicrobial activity exhibiting strong bactericidal action against *S. aureus*. The extracts were found to inhibit virulent enzymes that aid in *S. aureus* pathogenisis. The presence of therapeutically important phytochemicals coupled with its angiogenesis property suggests that it can be used as a wound healing agent.

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