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Development of Bio-Scaffold for the Treatment of 3º Burn Wounds

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ABSTRACT
Wound healing, as a normal biological process in the human body, is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. A better understanding of the influence of these factors on repair may lead to therapeutics that improve wound healing and resolve impaired wounds. In the present study we developed a gelatin-based bio scaffold for the treatment of wounds. Hemolytic assay was conducted to study the hemo-compatibility of bioscaffold towards the RBC cells. The results showed that the bioscaffolds were compatible towards the RBC cells with an IC 50 value of 124.2 µg/ml. Anti-inflammation assay exhibited anti-inflammation activity maximum at the concentration of 500 µg/ml with an IC 50 value of 51.02 µg/ml. The bioscaffold showed a significant antibacterial and antifungal activity against human microbial pathogens: such as Propionibacterium acnes, Proteus vulgaris, E.Coli, Staphylococcus aureus, Candida albicans and Aspergillus niger. The efficiency of sample Gelatin+sample against Propionibacterium acnes and Staphylococcus aureus found to be high at the concentration of 500 µg/ml and the zone of inhibition was found to be 10 mm. The maximum zone of inhibition against fungal species were found to be 7 mm against Candida albicans at the concentration of 500 µg/ml. Taken together, these results showed that the gelatin-based bio scaffold could be a promising approach for the treatment of wounds.
Keywords *Wound healing , bioscaffolds , Anti-inflammation assay*

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I. Introduction

Healthy skin, bone, and muscle undergo in situ self-healing that prevents the accumulation of defects from tissue aging and fatigue.. Healing and biomaterials are most commonly linked through the tissue response to an implant Wound healing is divided into inflammation, proliferation, and remodeling. Various studies have aimed at modulating inflammation and proliferation. Even though inflammation is a common event in allergic diseases, autoimmune diseases, infectious diseases, and others, it is essential for proper healing .

The objective of wound management is to heal the wound in the shortest time to prevent infection and minimize pain, discomfort, and scarring. The successful treatment of a wound should ensure that the amount of necrotic tissue is reduced and that microbial invasion is prevented . Traditional wound management used simple materials such as gauze according to the principle “to cover and conceal”. More recent developments have taken advantage of the deeper understanding of the underlying molecular and cellular mechanisms. Ensuring that the pharmacological, pharmacokinetic, and mechanical requirements of wound healing are met will provide a novel approach to remove the barriers to natural healing and enhance the effects of advanced therapies Wound infection is a manifestation of disturbed host–bacteria equilibrium in a trau-matized tissue environment in favor of bacterial proliferation. A wound infection can elicit a systemic response such as sepsis, but also inhibit any process of the wound healing cascade.

Antimicrobial biomaterials used for fabrication are especially interesting. Synthetic biomaterials offer many advantages over natural biomaterials as they can be synthesized and modified in a controlled manner according to the specific requirements needed to produce constant and homogenous physical and chemical properties as well as stability

II. Material And Methods

Composition of Poly herbal formulation (4 different plant samples)

Psidium guava-5gm, Aleo barbadensis - 20 gm , Curcuma langa - 15gm , Parapipotadenia- 10gm

Preparation for plant cold extraction

The powdered materials (four different plant samples) 10gm perextracted using 100ml of ethyl acetate and incubated at 4ºC for further investigation. The polyherbal extract was separated, primarily filtered by Muslin cloth followed by filtration with filter paper and Whatman No.1 paper and dried by rotary evaporator.

Preparation for gel formulation

About 1g of Gelatin was added to 100 µl samples and dissolved in 10ml of distilled water in a magnetic stirrer. Control was made by dissolving 1gm of Gelatin in 10ml of distilled water.

Swelling ratio

In order to measure the swelling degree of Gelatin scaffolds in comparison with template Gelatin membranes, the membrane samples were cut into pieces weighing 0.05g in an eppendorf which was taken as an initial weight. These dried samples were soaked in 1ml of distilled water, then the weight was taken at specific time intervals of initial (0), 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min when the water was completely removed. The values were noted down.

Hemolytic Assay

Human blood 5mL was collected from healthy volunteers in the tubes containing 3.8 % sodium citrate to prevent coagulation and washed three times in 9 volumes of sterile 0.9 % NaCl saline solution. After each washing, cells were pelleted by centrifugation at 1500 rpm for 5 min and the supernatant was discarded. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Erythrocytes suspension was prepared by adding 100 µL of erythrocytes in 900 µL 1XPBS. Each 100 µL erythrocytes suspension was mixed with 100 µL of test samples (Bioscaffold and Control) (500, 250, 100, 50 and 10 µg/mL) and 200 µL of 1XPBS was used as negative control and 100 µL of 1% SDS as positive controls to which 100 µL, each sample were replicated three times in 96 well plates. The volume of reaction mixture was made up to 300 µl by adding 1X PBS. The plates are incubated at 16℃ for 24 hours. Collect 100 µL of supernatant in another 96 well plate. Measure OD at 540nm using microtitre reader. The average value was calculated from triplicate assays. Hemolysis percentage for each sample was calculated by the formula,

$$\text{Hemolysis Percentage} = \text{Test OD} - \text{Negative OD} / \text{Positive OD} \times 100$$

Anti-inflammatory activity - Inhibition of albumin denaturation

Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakat *et al*. with slight modification. 500 µL of 1% bovine serum albumin was added to Gelatin+Sample and Control- Gealtin (500, 250, 100, 50 and 10 µg/mL) of test sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = 100 - ((A_1 - A_2)/A_0) \text{ X } 100)$$

Where A_1 is the absorbance of the sample, A_2 is the absorbance of the product control and A_0 is the absorbance of the positive control.

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Antibacterial Activity

Petri plates containing 25 ml nutrient agar medium were seeded with 24hr culture of bacterial strains (*Staphylococcus aureus* and *Escherichia coli*). Wells were cut and different concentration of samples Gelatin+ sample and Control- Gelatin (500 µg/ml, 250 µg/ml, 100µg/ml, and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells.

Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

Antifungal activity

Petri plates containing 25ml potato dextrose agar medium were seeded with 24hr culture of fungal strain *Aspergillus niger*and *Candida albicans*. Wells were cut and different concentration of sample Gelatin+ sample and Control- Gelatin (500 µg/ml, 250 µg/ml, 100µg/ml and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

Moisture content

The moisture content of the Bioscaffold sample was determined by the following method suggested by Tandon (2005) with little modifications. The sample 0.10 g was transferred into a sterilized Petri dish. The Petri dish was closed with a lid and kept in a hot air oven at 50˚C for four hours. Then, it was cooled in desiccators and weighed. The loss in weight represents the moisture content of the samples.

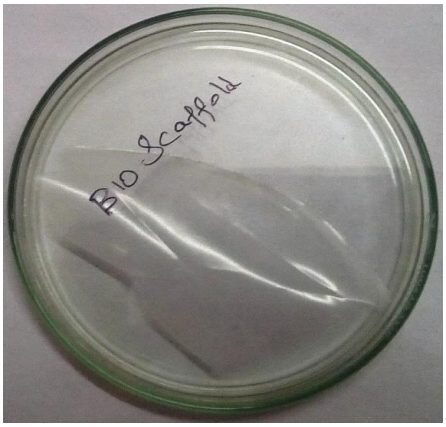
III. Results

Wound healing refers to a living organism's replacement of destroyed or damage tissue by a newly produced. Wound dressing has been fabricated out of different type of biomaterials used. To alleviate and treat chronic wounds, various biomaterials have been developed. Among them, polymeric hydrogels have been widely used as a promising wound care material due to their beneficial properties, including solgelphase transition, moisturizing effect on the surrounding environment, biocompatibility, and structural similarity to the native extracellular matrix. The development of bioactive hydrogels that provide artificial cellular micro environments or stimulate surrounding tissues through physicochemical and biological stimuliisane merging trend in the fabrication of hydrogels.

The swelling degree was measured for all the samples. The swelling ratio of the membrane was at the maximum level at 40 min time interval and the bioscaffold exhibited highest swelling ratio. The hemo-compatibility assay was used to determine the hemolytic effect of bioscaffold and the results showed the hemo-compatibility effect. ML based scaffolds, demonstrated antibacterial activity against *Staphylococcus aureus, Proteus vulgaris, Propionibacterium acnes* and *Escherichia coli.* The activity was analyzed against these microbes showed a statistically significant inhibition at the concentration of 500 µg/ml. The antifungal activity of bio scaffold against *Aspergillus niger* and *Candida albicans* were tested which showed the maximum zone of inhibition of 7 mm at the concentration of 500 µg/ml.

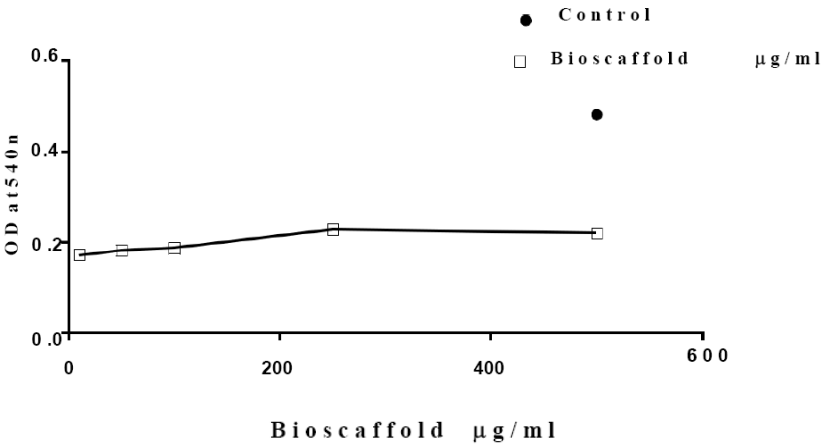
Notably, this study the demonstrated the antimicrobial activity, anti-inflammatory activity, hemo-compatibility and noncytotoxic to the human cells. These results confirm that the bio scaffold showed better wound healing effect and can be used for the treatment of 3rd degree burn wounds

Images for Bioscaffol



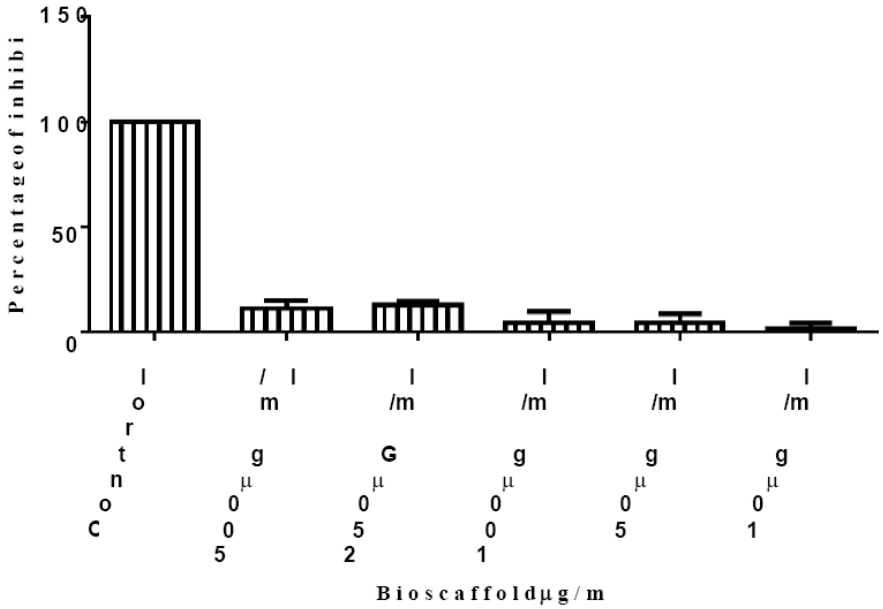
Hemolytic Assay
OD Value at 540 nm
Negative Control Mean OD value: 0.165
Positive Control Mean OD value: 0.481

S. No	Tested sample concentration (µg/ml)	OD Value at 540 nm (in triplicates)		
1.	Negative Control	0.142	0.155	0.198
2.	Positive Control	0.490	0.447	0.508
3.	500 µg/ml	0.230	0.200	0.229
4.	250 µg/ml	0.233	0.233	0.219
5.	100 µg/ml	0.182	0.166	0.215
6.	50 µg/ml	0.195	0.148	0.202
7.	10 µg/ml	0.161	0.167	0.189



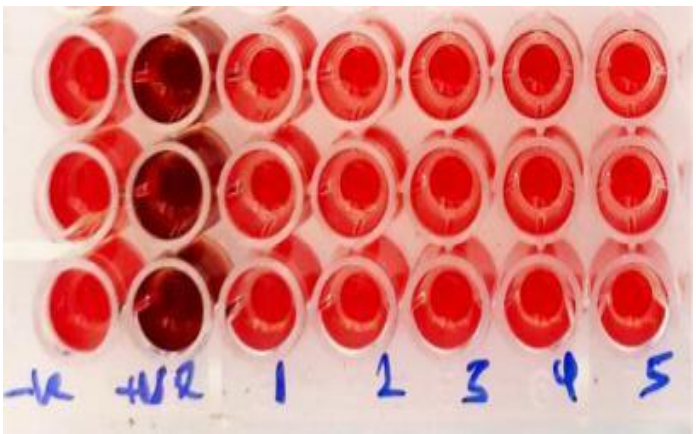
Percentage of Hemolysis (%)

S. No	Tested sample concentration (µg/ml)	Percentage of Hemolysis (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	13.51	7.27	13.30	11.36
3.	250 µg/ml	14.13	14.13	11.22	13.16
4.	100 µg/ml	3.53	0.20	10.39	4.70
5.	50 µg/ml	6.23	0	7.69	4.64
6.	10 µg/ml	0	0.41	4.98	1.79



C.IC50 Value of tested sample: 124.2 µg/ml

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		2.094
HillSlope		3.591
IC50		124.2
Std. Error		
LogIC50		0.1140
HillSlope		2.874
95% Confidence Intervals		
LogIC50		1.848 to 2.340
HillSlope		-2.616 to 9.798
IC50		70.45 to 218.9
Goodness of Fit		
Degrees of Freedom		13
R square		0.6001
Absolute Sum of Squares		14519
Sy.x		33.42
Number of points		
Analyzed	3	15

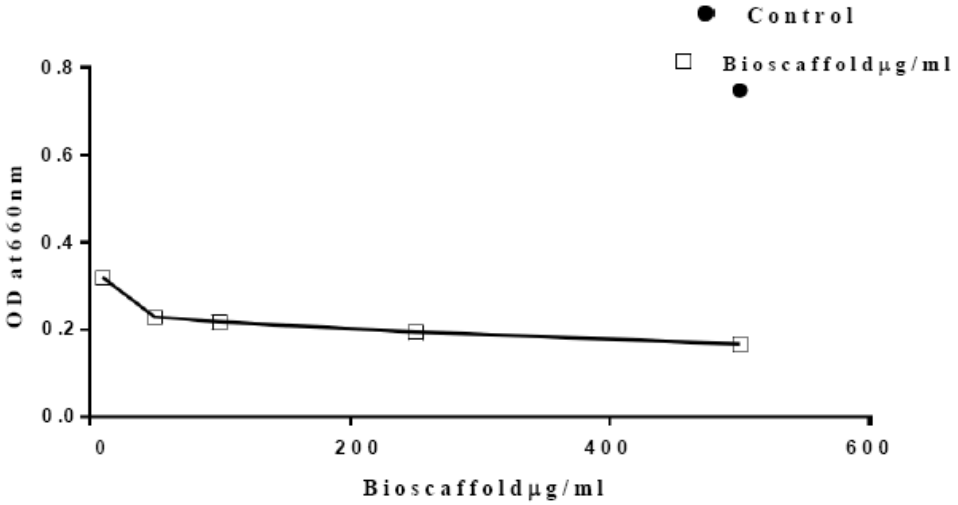


Anti-inflammatory activity - Inhibition of albumin denaturation A.

OD Value at 660 nm

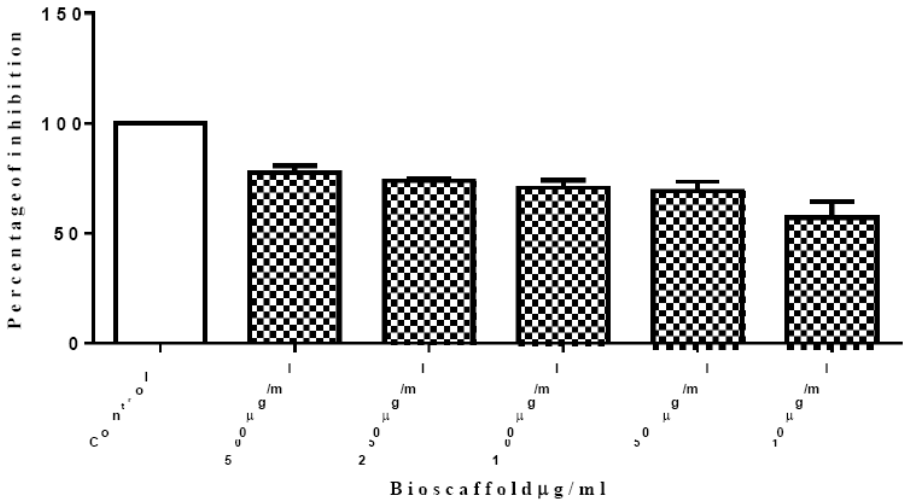
Control Mean OD value: 0.746

S. No	Tested sample Concentration (µg/ml)	OD Value at 660 nm (in triplicates)		
1.	Control	0.739	0.744	0.756
2.	500 µg/ml	0.183	0.140	0.179
3.	250 µg/ml	0.198	0.202	0.187
4.	100 µg/ml	0.219	0.243	0.193
5.	50 µg/ml	0.206	0.217	0.266
6.	10 µg/ml	0.283	0.294	0.382



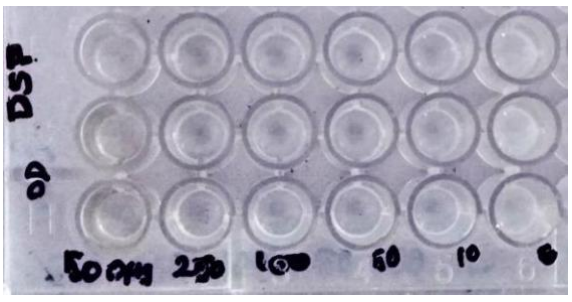
B. Inhibition percentage of albumin denaturation (%)

S. No	Tested sample concentration (µg/ml)	Inhibition percentage of albumin denaturation (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	75.46	81.23	76.00	77.65
3.	250 µg/ml	73.45	72.92	74.93	73.76
4.	100 µg/ml	70.64	67.42	74.12	70.72
5.	50 µg/ml	72.38	70.91	64.34	69.21
6.	10 µg/ml	62.06	60.58	48.79	57.14



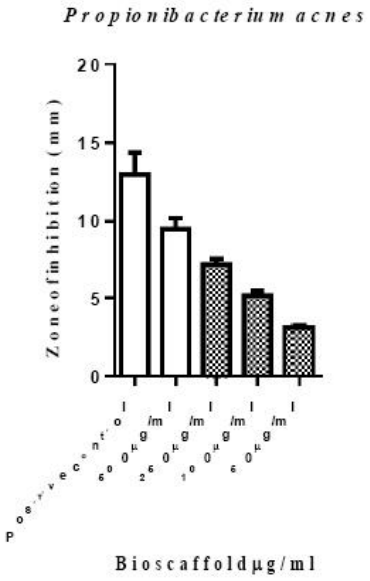
C. IC50 Value of tested sample: 51.02 µg/ml

log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.708
HillSlope		-1.346
IC50		51.02
Std. Error		
LogIC50		0.1149
HillSlope		0.4645
95% Confidence Intervals		
LogIC50		1.460 to 1.956
HillSlope		-2.349 to -0.3426
IC50		28.81 to 90.35
Goodness of Fit		
Degrees of Freedom		13
R square		0.7495
Absolute Sum of Squares		5402
Sy.x		20.39
Number of points		
Analyzed	3	15

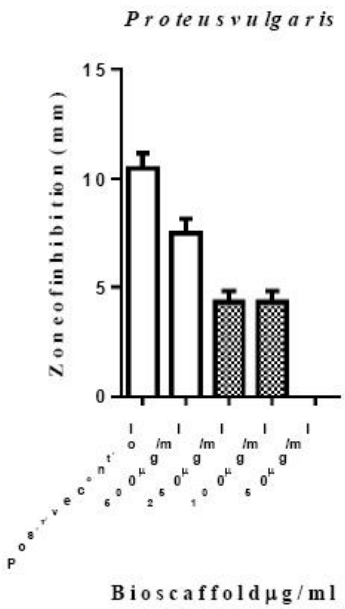


Antibacterial activity

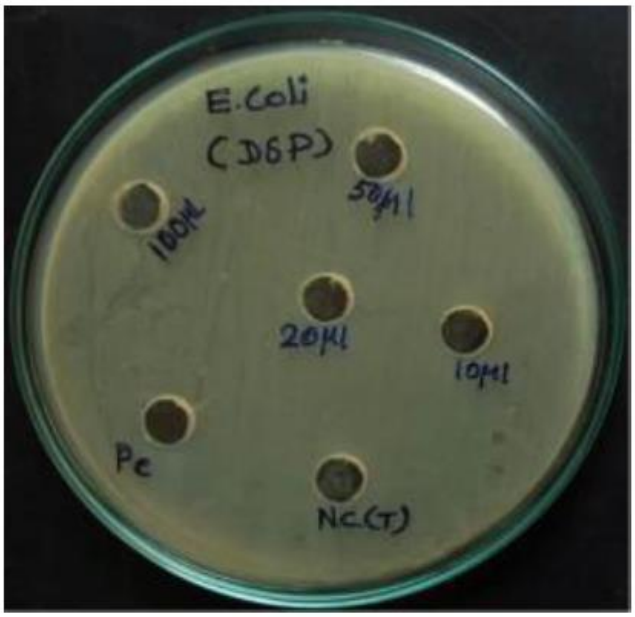
1.Effect of sample Bioscaffold against *Propionibacterium acnes.*



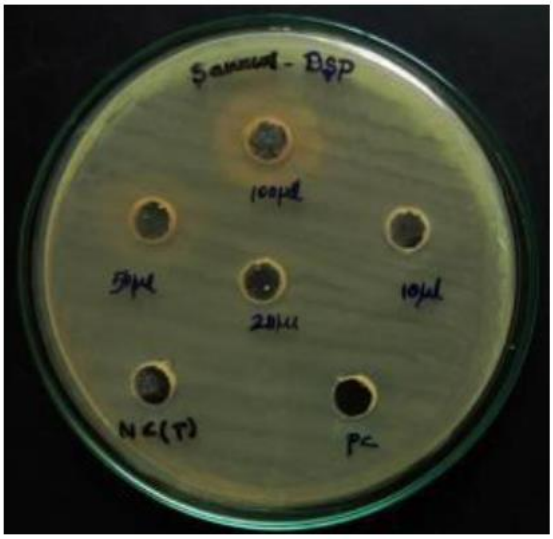
2.Effect of sample Bioscaffold against *Proteus vulgaris*.

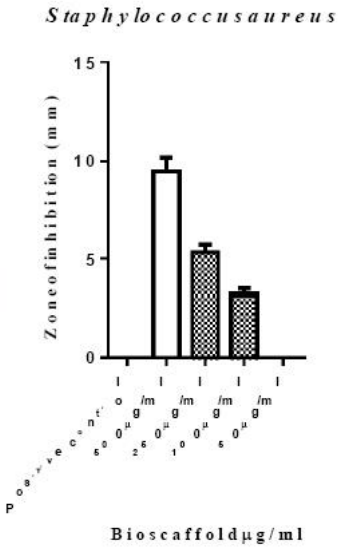


3.Effect of sample Bioscaffold against *E.Coli.*



4.Effect of sample Bioscaffold against *Staphylococcus aureus.*



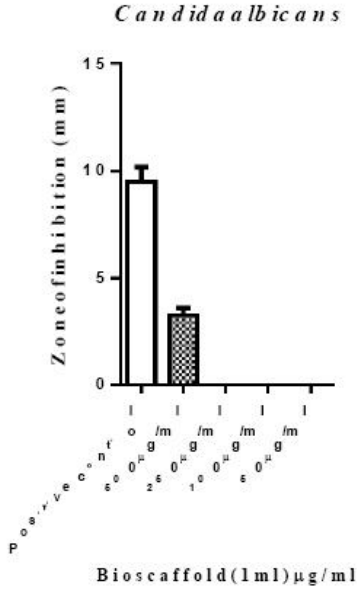


Means of zone of inhibition obtained by sample Bioscaffoldagainst *Propionibacterium acnes, Proteus vulgaris, E.Coli***and** *Staphylococcus aureus*.

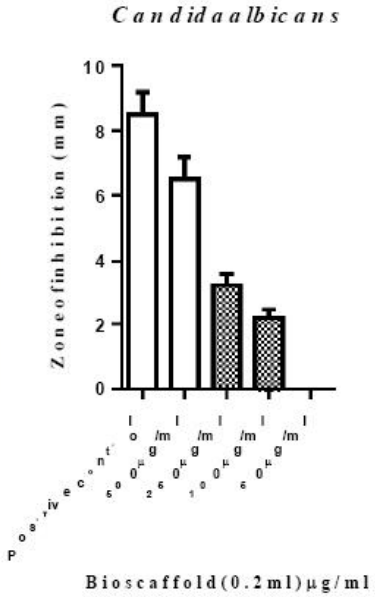
S.No	Name of the test sample	Name of the microorganism	Zone of inhibition(mm)				
			500 µg/ml	250 µg/ml	100µg/ml	50µg/ml	PC
1.	*Bioscaffold*	*Propionibacterium acnes*	10±1.0	7.5±0.5	5.4±0.4	3.2±0.2	14±2.0
2.		*Proteus vulgaris*	8±1.0	4.7±0.7	4.7±0.7	0	11±1.0
3.		*E.Coli*	0	0	0	0	4.5±0.5
4.		*Staphylococcus aureus*	10±1.0	5.6±0.6	3.4±0.4	0	0

Antifungal activity

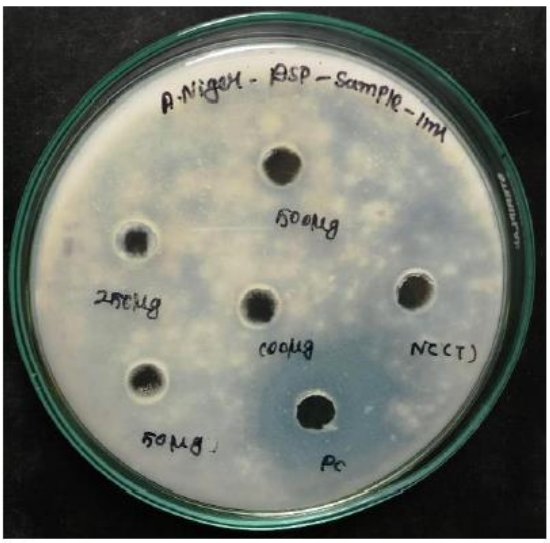
1.Effect of sample Bioscaffold (1ml) against *Candida albicans.*



2.Effect of sample Bioscaffold (0.2ml) against *Candida albicans.*



3.Effect of sample Bioscaffold (1ml) against *Aspergillus niger.*



4.Effect of sample Bioscaffold (0.2ml) against *Aspergillus niger.*

Table . Means of zone of inhibition obtained by sample Bioscaffold (1ml and 0.2ml) against *Aspergillus niger and Candida albicans*.

S.No	Name of the test Sample	Name of the test sample	Zone of inhibition(mm)				
			500 µg/ml	250 µg/ml	100µg/ml	50µg/ml	PC
1.	*Aspergillus niger*	Bioscaffold (1ml)	0	0	0	0	12±1.0
2.		Bioscaffold (0.2ml)	0	0	0	0	13±1.0
3.	*Candida albicans*	Bioscaffold (1ml)	3.5±0.5	0	0	0	10±1.0
4.		Bioscaffold (0.2ml)	7±1.0	3.5±0.5	2.4±0.4	0	9±1.0

IV. CONCLUSION

Scaffold was prepared from herbal formulation for the 3rd degree burn wounds, by examining the results of swelling ratio, hemolytic assay, antibacterial, antifungal activity and anti-inflammatory activity. Scaffold membranes prepared and notable difference in the results when comparing gelatin with membrane prepared by combination of gelatin and polyherbal formulation. The swelling ratio determines the absorption capacity of the membranes and so when applied in wound it could swell up aids in better regeneration of tissue. The membranes were compatible and caused to a smaller amount of lysis of red blood cells. They exhibited antibacterial and antifungal activity against common pathogenic microbes present in the skin of mammals. Thus, it is suitable for 3rd degree burn wounds. Future studies can be carried out invivo using animal models like albino rats.

REFERENCE

[1]. Afsharian, Y. P., and Rahimnejad, M. (2020). Bioactive electrospun scaffolds for wound healing applications: A comprehensive review. Polymer Testing, 106952.

[2]. Alexandra Elena Stoica, Cristina Chircov and Alexandru Mihai Grumezescu (2020).Hydrogel Dressings for the Treatment of Burn Wounds: An Up-To-Date Overview, Materials, 13, 2853.

[3]. Alharbi, Andrzej Piatkowski, Rolf Dembinski, Sven Reckort, Gerrit Grieb, Jens Kauczok and Norbert Pallua (2012). Treatment of burns in the first 24 hours: simpleand practical guide by answering 10 questionsin a step-by-step form, World Journal of Emergency Surgery, 7:13

[4]. Ali, A.; Garg, P.; Goyal, R.; Kaur, G.; Li, X.; Negi, P.; Valis, M.; Kuca, K.; Kulshrestha, S.A(2021). Novel Herbal Hydrogel Formulation of Moringa oleifera forWound Healing. Plants 10, 25.

[5]. Arthur k. C. Li, B.Chir, (eng.), H. Paul Ehrlich, Robert. Trelstad, Mary Jo Korol (2014). Differences in Healing of Skin Wounds Caused by Burn and Freeze Injuries, Lippincott Company, Vol. 191.9 No. 2.

[6]. Atul AC, KomalVig, Baganizi DR, Sahu R, Dixit S, Dennis V, Ram Singh S, and Shree kumar RP, (2016). Future Prospects for Scaffolding Methods and Biomaterials in Skin Tissue Engineering: A Review. Int J Mol Sci. 17(12).

[7]. Ayyanar M, Ignacimuthu (2009). Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences, International Journal of Applied Research in Natural Products,Vol. 2(3), pp. 29-42.

[8]. Bairy. K.L. (2002). Wound healing potentials of plant products, Journal of Natural Remedies, Vol 2/1, 11 – 20.

[9]. Bhatia, A., O'Brien, K., Chen, M., Wong, A., Garner, W., Woodley, D. T., and Li, W. (2016). Dual therapeutic functions of F-5 fragment in burn wounds: preventing wound progression and promoting wound healing in pigs. Molecular Therapy - Methods and Clinical Development, 3, 16041.

[10]. Biliaderis, C., (1992). Structure and phase transition of starch in food systems. Food Technol. 46(6), 98–109.

[11]. Brochu, A.B., Craig, S.L., (2011). Self-healing biomaterials. J. Biomed. Mater. Res. A 96 (2), 492–506.

[12]. Cima LG, Vacanti JP, Vacant C, Ingber D, Mooney D, and Langer R, (1991). Tissue engineering by cell transplantation using degradable polymer substrates. J. Biomech. Eng. 113, 143-151.

[13]. Dai, T., Tanaka, M., (2011). Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert Rev. Anti Infect. Ther. 9 (7), 857–879.

[14]. De Moraes, J. M., Eterno de Oliveira Mendonça, D., Moura, V. B. L., Oliveira, M. A. P., Afonso, C. L., Vinaud, M. C., de Souza Lino, R. (2012). Anti-inflammatory effect of low-intensity laser on the healing of third-degree burn wounds in rats. Lasers in Medical Science, 28(4), 1169–1176.

[15]. Dhandayuthapani B, Yoshida Y, Maekawa T, and Shakthi kumar D. (2011). International Journal of Polymer Science. Pp19.

[16]. Dons, T., and Soosairaj, S. (2018). Evaluation of wound healing effect of herbal lotion in albino rats and its antibacterial activities. Clinical Phytoscience, 4(1).

[17]. Dorai, A. (2012). Wound care with traditional, complementary and alternative medicine. Indian Journal of Plastic Surgery, 45(2), 418.

[18]. Garcia-Espinoza JA, Aguilar-Aragon VB, Ortiz-Villalobos EH, Garcia-Manzano RA, Antonio BA (2017) Burns: Definition, Classification, Pathophysiology and Initial Approach. Gen Med (Los Angeles) 5: 298.

[19]. Guerra, L., Dellambra, E., (2009). Tissue engineering for damaged surface and lining epithelia: stem cells, current clinical applications, and available engineered tissues. Tissue Eng. Part B Rev. 15 (2), 91–112.

[20]. Hardy Schwarze, Markus Kuntscher, Christian Uhlig, Helmut Hierlemann(2008).Suprathel, a New Skin Substitute, in the Management of Partial-Thickness Burn Wounds, Annals of Plastic Surgery, Volume 60, Number 2.