Research Article

EVALUATION OF ANTIBACTERIAL EFFICACY AND WOUND HEALING ACTIVITY OF NATURAL FIBRE BASED WOUND DRESSING COATED WITH NATURAL EXTRACTS OF *CALOTROPIS GIGANTEAN*, *EUCALYPTUS GLOBULES* AND BUDS OF *SYZYGIUM AROMATICUM* ENHANCED WITH EPIDERMAL GROWTH FACTOR

Ramesh P ^{1*}, Prakash C ², Sukumar N ³, Palaniswamy N K ⁴ and Sengottuvelu S ⁵

¹Department of Textile Technology, K S Rangasamy College of Technology, Tiruchengode, India

²Department of Fashion Technology, Sona College of Technology, Salem, India

³Department of Textile Engineering, Wollo University, Ethiopia

⁴Department of Textile Engineering, Aksum University, Ethiopia

⁵Department of Pharmacy, Nandha College of Pharmacy, Erode, India

*Corresponding Author Email: p.ramesh05@gmail.com

Article Received on: 25/04/17 Approved for publication: 28/05/17

DOI: 10.7897/2230-8407.08581

ABSTRACT

In this work, the leave extracts of *Calotropis gigantean*, *Eucalyptus globules* and buds of *Syzygium aromaticum* were prepared and coated on natural fibres based bamboo gauze fabric for wound dressing applications. The fabrics coated with different compositions of the natural extract were enhanced with rhEGF (REGEN-D™ 60). The antibacterial activity of the developed samples was studied after the identification of its mechanical properties and Scanning Electron Microscopy. The wound healing efficacy of the coated fabric samples were studied through *In vivo* method using Wister Albino rats. The type of wound selected for the study was second degree burn wound. The dorsal surface of the rat's skin was removed and second degree burn wound was created. Then the developed fabric samples were dressed over the wound surface and the wound healing was studied by wound closure rate in equal interval of days. A specified composition of natural extracts coated on bamboo gauze fabric enhanced with rhEGF (REGEN-D™ 60) exhibits good antibacterial property was confirmed from the formation of zone of inhibition in antibacterial test against Gram-negative (*Escherichia coli*) and a Gram-positive bacterium (*Staphylococcus aureus*) and appropriate wound closure rate on second degree burn wound through *In vivo* evaluation. Hence therefore it was concluded that coated bamboo gauze fabrics can be suitable for faster burn wound healing process.

Keywords: Wound dressing, *Calotropis gigantean*, *Eucalyptus globules*, *Syzygium aromaticum*, rhEGF, In Vivo.

INTRODUCTION

The disruption on the natural anatomical structure results in the formation of wound ^{1,2,3} and depending upon their tissue loss wounds can be classified into two types i.e., wounds without tissue loss (e.g. in surgery) and wounds with tissue loss (which includes burn wounds, wounds caused by trauma and diabetic ulcers). To cover the wounds and absorb bleeding these wound dressings have been used⁴.

The flow of wound healing includes like embryogenesis and tissue regeneration were fundamental processes. It includes several individual but inter connected stages like homeostasis, inflammation stage, proliferation and matrix remodeling^{5,6}.

Burn wounds can be classified (Figure 1 – Source – The Burn Patient, E.James Radin) according to involvement of skin and deeper tissues as follows:

First-degree burn or epithelial burns – Skin is erythematous without vesication.

Second-degree burns – Involving epidermis and variable thickness of dermis. This is again divided into

- **Second-degree superficial** –where vesication and inflammation is seen in skin as only papillary dermis is involved.

- **Second-degree deep** –eschar formation is seen as it involves deep reticular dermis.

Third-degree burn – Also known as full thickness burns – eschar formation is present in these burns.

Figure 1: Degree of Burn Wounds

Wound dressings are used to encourage the various stages of wound healing and create better healing conditions for said healing. The plan of wound care should always concentrate on using the appropriate wound dressing material and treatments to reduce dressing frequency. An ideal wound dressing material should provide a suitable environment to the wound dressing interface, as well as mechanical and bacterial protection that allows gaseous and fluid exchanges⁷.

During burn wound healing process, the dressing protects the injury and contributes to the recovery of epidermal tissues. Due to biocompatibility, biodegradability and similarity to macromolecules recognized by the human body, some natural occurring polymers such as polysaccharides (alginates, chitin, chitosan, heparin, chondroitin), proteoglycans and proteins (collagen, gelatin, fibrin, keratin, silk fibroin, egg shell membrane) are extensively used in wounds and burns management.

Calotropis is used as traditional medical plant with unique properties. Traditionally it is used alone or with other medicinal to treat common disease such as fever, indigestion, cough, cold, asthma, vomiting, diarrhea. According to ayurveda dried while plant is a good tonic, expectorant, depurative and anthelmintic. The dried fibre is substitutes for ipecacantha. The fiber is febrifuge anthelmintic, depurative, expectorant and laxative⁸.

Eucalyptus species is an important source of many pharmacologically and medically important chemical used for various activities like analgesic, antifungal, anti-inflammatory, anti-bacterial, anti-diabetic, anti-oxidative, cytochrome p450 enzymes inhibitor, anti-viral, anti-tumor, anti-cancer cytochrome p450 inhibitor and hepatoprotective properties⁹.

Syzygium aromaticum contain much properties like anti-fungal, anti-viral, anti-microbial, anti-diabetic, anti-inflammatory, anti-thrombotic anesthetic. *Syzygium aromaticum* represents one of the natural antiseptic which consists of Eugenol which is used for various medical purposes¹⁰.

MATERIALS AND METHODS

Bamboo yarn (100 %) of Ne 30s was sourced from S.V.TEX, Tirupur, Tamil Nadu, India and it was woven to gauze fabric. Leaves of calotropis gigantea were sourced from sadhumugai, Sathyamangalam. Leaves of eucalyptus globulus were sourced from orange groove estate, ootacamund. The buds of *Syzygium aromaticum* were sourced from a departmental store in Erode. The recombinant human Epidermal Growth Factor (rhEGF) as REGEN-D™ 60 was purchased from M/s. Bharat Biotech International Limited, Hyderabad, India. Deionized water was used for all experiments.

Preparation of plant extracts

The collected fresh and healthy leaves of *Calotropis gigantea* (CG), *Eucalyptus globules* (EG) and buds of *Syzygium aromaticum* (SA) were washed with plain water. Then the leaves and buds were washed by distilled water and it was subjected to shade dry. The dried leaves and buds were grinded into a fine powder which is used for the study. The extraction process was carried out by taking each 3g of dried powder and mixed with 50mL of 80% methanol. The container was closed completely and left ideal for overnight. After overnight of incubation, the extract was filtered through filter paper and to concentrate the extract, it was evaporated at room temperature. This methanol extract was used for the application on fabrics.

The filtered liquid was measured and the pH of the solution was evaluated using pH meter or pH paper.

Finishing of Fabrics

Calculated amount of extracts were taken as per the ratio of 20:20:20, 30:20:20, 20:30:40 in the order of EG: CG: SA (Table 1). The solution was taken as per the ratio and mixed thoroughly. The samples to be coated were put in to distilled water and were boiled at the temperature of 60°C for about ½ an hour. The boiled samples were taken out and the samples were put into extracted solution of predetermined ratio. The samples were coated with the extract and it was tend to padding mangle. The padded samples were spread on a dry surface and calculated amount of prepared drug solution (5gpl of REGEN-D™ 60 (Burn) in 100 ml of distilled water) were coated over the samples. Then the samples were dried in oven at 60°C.

Antibacterial Efficacy test

As per AATCC 147 standard antibacterial activity was assessed. In this study two types of bacteria were used namely Gram-negative (*Escherichia coli*) and a Gram-positive bacteria (*Staphylococcus aureus*). The line of incubation of antimicrobial agent was shown by the presence of growth inhibition zones measured by using Muller- Hinton (HiMedia)^{3,11}.

Fabric Properties

The bamboo gauze fabric specifications such as ends per inch, picks per inch, warp crimp and weft crimp were identified and cover factor of the fabric was calculated. As per the ASTM D1777-96 and ASTM D6828 standard methods, the thickness and stiffness of the samples were determined respectively. The areal density of the fabric was measured using GSM cutter method as per ASTM D3775¹².

Scanning Electron Microscopy Analysis

The coated samples were studied by scanning electron microscope (SEM, JEOL, JSEM-6390LV, Japan) for its Surface Morphology and cross – section.

Animal Test Methods

Wister Albino Rats of initial weight 150.71 ± 5 gm were taken for evaluation. The rats were found to be hygienic and followed to have proper food at regular interval and have proper digestion the life cycle of the rats have been monitored. The rats were weighed before and after treatment of wound healing process. All animal procedures were according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and Institutional Animal Ethics Committee (IAEC) approved by the Nanda College of Pharmacy, vide proposal No.NCP/IAEC/No: 8/2014 -15. Total of 30 rats were taken for study and they were divided into six groups.

Wound Creation

Wister Albino Rats were given anesthetic of minimum level of 0.05 ml and the hairs of the rats were shaved using shaver in position of lateral half way between the midline and it was left for 45min. A metal rod of 2cm diameter was heated to 90°C in boiling water for 15min and it was sterilized. Then the metal rod was taken and cooled down to 60°C and second degree wound was made on individual rats with the same temperature and

pressure and the rats were allowed to take rest for about one day. The group I rats were not treated with any of the material and left open for natural healing. The group II rats were commercially available drug Silver sulphadiazine (0.5g of 1%) cream for every two days. The group III rats were treated with developed sample NES (Table I). The group IV, V & VI rats were treated with the developed samples NES1, NES2 & NES3 respectively for every three days.

Wound Healing Observation

Treated wounds were subjected to periodical analysis on 0th, 3rd, 6th, 9th, 12th and 15th day. The wound healing rate was observed with the reduction in wound size. The size of the wound was measured by tracing with transparent butter paper. With Nikon digital camera (Model: Nikon Coolpix S6700 Point & Shoot Camera) the photography of wound closure was captured at the time interval mentioned earlier. The percentage of wound closure was calculated by the initial and final area using graph paper during observation. The wound size reduction was calculated as follows:

$$\% \text{ of Wound size reduction} = (D_i - D_f) / D_i \times 100$$

Where D_i was the initial area of wound on 0th day and D_f was the area of wound at the time of treatment with developed material on 3rd, 6th, 9th, 12th and 15th day accordingly.

RESULTS

Properties of Gauze fabric

The mechanical properties of bamboo gauze fabric were measured before and coating of the natural extracts. The findings of the fabric properties are tabulated below Table 2.

Antibacterial Efficacy test

The anti-bacterial activity of the coated samples was tested against *Escherichia Coli* and *Staphylococcus aureus* by agar diffusion method. The results of anti-bacterial activity of the coated samples was tabulated in Table 3 and the captured images of formation of zone of inhibition were shown in figure 2.

Morphological Analysis

In this study, the surface morphology of the natural extract coated samples was studied with SEM. The important parameters of a natural extract coated material used for wound healing applications were the microstructure and architecture of the wound dressing material. These parameters were completely studied with the images observed in SEM shown in figure 3.

In vivo Evaluation

The observations of the wound healing rate in the duration as mentioned earlier of the coated samples were shown in figure 4.

Table 1: Preparation ratio of *Calotropis gigantean*(CG), *Eucalyptus globules* (EG), buds of *Syzygium aromaticum*(SA) and rhEGF

Group	100 % Bamboo Yarn			
Sample	NES	NES1	NES2	NES3
CG (ml)	20	20	30	20
EG (ml)	20	20	20	30
SA (ml)	20	20	20	40
rhEGF (µg)	-	5	10	15
REGEN-D™ 60 (Burn)	-	-	-	-

Table 2: Mechanical Properties of Bamboo Gauze fabric

S n	Properties	Before Coating	After Coating			
			NES	NES1	NES2	NES3
1	Weave	Plain				
2	Ends/inch(EPI)	34				
3	Picks/inch(PPI)	28				
4	Areal Density	50.7 g/m ²	50.7 g/m ²	50.7 g/m ²	50.7 g/m ²	50.7 g/m ²
7	Thickness mm)	0.03 mm	0.03 mm	0.03 mm	0.03 mm	0.03 mm
8	Flexural Rigidity (mg.cm)					
	Warp -	84.83.	95.29	95.28	95.31	95.26
	Weft -	72.23	83.15	83.16	83.16	83.14

Table 3: Results of Antibacterial Test against gram negative and gram positive bacteria

S.N	Samples	Name of Organisms (Zone of Inhibition in diameter (mm))	
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1	Untreated fabric	0	0
2	Control group	20	18
3	NES	10	8
4	NES 1	6	7
5	NES 2	8	7
6	NES 3	13	11

Figure 2: (a) Formation of zone of inhibition against *Staphylococcus aureus* and (b) Formation of zone of inhibition against *Escherichia coli*

Figure 3: SEM micrographs of the coated Bamboo Gauze fabrics

Figure 4: Wound Closure Study

DISCUSSION

The results of mechanical properties of the natural extract coated fabric samples shows that the areal density of the fabric increases with 1.3 % after coating. Similarly, the result clearly shows the treated samples have a slight difference in the stiffness comparatively to the untreated samples.

The test results of antibacterial activity of the natural extract coated samples were comparatively analyzed as shown in the Figure 2. The formation of zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* in the sample NES 3 which is about 13 mm and 11 mm after 24 hrs of inhibition. On its consolidation NES 3 shows better antibacterial property which was conformed with the evident from the zone of inhibition as shown in figure 2. It also concludes that when the ratio of natural extracts & rhEGF was increased the better will be the antibacterial property.

The morphological study of coated fabric as shown in figure 3 reveals that natural extracts coated penetrates evenly over the surface of the gauze fabric samples. The porous nature of the fabrics was not affected, was concluded from the images investigated after coating with the natural extract on the gauze fabric.

The wound healing rate was compared through *In vivo* evaluation and shown in figure 4. The results confirmed that sample NES 3 has faster wound closure rate. The images of wound size reduction after treatment with natural extract coated wound dressing on 0th, 3rd, 9th and 15th days were shown in figure 4. The size reduction of wounds in sample NES 3 shows faster wound healing rate when compared with other compositions (Wounds not treated, treated with commercially available drug Silver sulphadiazine and other ratios of *Calotropis gigantean*(CG), *Eucalyptus globules* (EG) and buds of *Syzygium aromaticum* (SA) enhanced with rhEGF (REGEN-D™ 60 (Burn)

CONCLUSION

It is found that the different natural extracts were coated in all the samples evenly. The samples coated with natural extracts exhibit good anti – bacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The results of In – Vivo evaluation reveals that, the coated gauze fabrics were suitable for dressing the burn wounds and also it gives a better wound healing rate. It was also further proven that when ratio of the natural extracts was increased the wound healing rate was increased.

ACKNOWLEDGEMENT

The authors are thankful to K.S.Rangasamy College of Technology, Tiruchengode, Namakkal District, Tamil Nadu, India for their technical support and constant encouragement.

REFERENCES

1. Lazarus GS, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. Archives of Dermatology Journal. 1994;130 :489-493.
2. Priyanka Agrawal, Sandeep Soni, Gaurav Mittal, and Aseem Bhatnagar. Role of Polymeric Biomaterials as Wound Healing Agents. The International Journal of Lower Extremity Wounds 2014; 13(3): 180– 190.
3. Ramesh P, Prakash C, Palaniswamy N K, Sukumar N and Sengottuvelu S . Development and characterization of bamboo based wound dressing coated with natural extracts of curcumin, aloe vera and chitosan enhanced with recombinant human epidermal growth factor and *In vivo* evaluation for wistar albino wounded rats. International Research Journal of Pharmacy 2017; 8(3): 50 – 55.
4. Shuk-fan Tong, Joanne Yip, Kit-lun Yick and Chun-wah Marcus Yuen. Exploring use of warp-knitted spacer fabric as a substitute for the absorbent layer for advanced wound dressing. Textile Research Journal 2015; 85(12): 1258–1268.
5. Diegelmann RF, Evans MC. Wound Healing: an overview of acute, fibrotic and delayed healing. Frontiers in bioscience Journal. 2004; 9: 283- 289
6. Kanhaiya Singh, Neeraj K. Agrawal, Sanjeev K. Gupta, Gyanendra Mohan, Sunanda Chaturvedi and Kiran Singh. Differential Expression of Matrix Metalloproteinase-9 Gene in Wounds of Type 2 Diabetes Mellitus Cases With Susceptible -1562C>T Genotypes and Wound Severity. The International Journal of Lower Extremity Wounds 2014, 13(2) 94– 102
7. Altman, GH, Diaz, F, Jakuba, C, Calabro, T, Horan, RL, Chen, J, Lu, H, Richmond, J & Kaplan, DL, Silk-based biomaterials, Biomaterials, 2003, 24, (3), 401-416
8. Ajay Kumar Meena, Ajay Yadav, M M Rao, Ayurvedic uses and pharmacological activities of *Calotropis procera* Linn. Asian Journal of Traditional Medicines, 2011, 6 (2), 45 – 53
9. Hardel Danendra Kumar, Sahoo Laxmidhar, A Review of Phytochemical and pharmacological of *Eucalyptus Globulus*: A Multipurpose Tree, International Journal of Research in Ayurveda & Pharmacy, 2011, 2 (5), 1527 – 1530.
10. Parle Milind and Khanna Deepa, Clove: A Champion Spice, International Journal of Research in Ayurveda & Pharmacy, 2011, 2 (1), 47 -54.
11. N Sukumar, T Ramachandran and CB Lakshmikantha, Development and characterization of cactus-dextrin-recombinant human epidermal growth factor based silk scaffold for wound dressing applications, Journal of Industrial Textiles, 2012; 43, 565–576
12. O L Shanmugasundaram, Development and characterization of cotton and organic cotton gauze fabric coated with biopolymers and antibiotic drugs for wound healing, Indian Journal of Fibre & Textile Research, 2012, 37, 146 – 150.
13. E.James Radin, The Burn Patient, Available from: www.srems.com/site/cme/BurnPatient.pdf.

Cite this article as:

Ramesh P et al. Evaluation of antibacterial efficacy and wound healing activity of natural fibre based wound dressing coated with natural extracts of *Calotropis gigantean*, *Eucalyptus globules* and buds of *Syzygium aromaticum* enhanced with epidermal growth factor. Int. Res. J. Pharm. 2017;8(5):95-100 <http://dx.doi.org/10.7897/2230-8407.08581>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.