

Development of Antimicrobial Wound Dressing Bandage using *Chromolaena Odorata* Leaf Extract

T. H. Sukirtha¹, Mohanadoss Ponraj², Aruna U¹

Department of Microbiology, Nehru Arts and Science College, Coimbatore, India¹

Department of Biological Sciences²

School of Mathematics and Natural Sciences, The Copperbelt University, Kitwe, Zambia²

Corresponding Author: goldking1977@gmail.com²

Abstract: A bandage is a standard of biomaterial used on wounds to protect from infections and to cure wounds. The adhesive bandage also known as sticking plaster protects the wound from friction, bacteria, damage, and dirt. In this study, the leaf extracts of plant (*Chromolaena odorata*) coated on textiles are being used as a potential drug to promote wound healing. The phytochemical screening and antimicrobial activities of the ethanolic plant leaf extracts was carried out. The leaf extracts were coated on non-woven viscose rayon fabric using Pad-dry cure method. The physical and biological parameters of the coated fabric were analyzed to determine the process of wound healing. The parameters of pH and absorbency were evaluated using the standard protocols obtained from medical textiles. The antimicrobial efficiency (standard protocol AATCC 100), cytotoxicity (MTT assay) and in vitro scratch wound assay using cell lines was studied. The results showed that developed herbal coated bio-bandage featured all the characteristics for ideal dressing. Therefore, it can be promoted as novel bio-bandage for the healing of wounds.

Keywords: Phytochemical, Bio-bandage, Cytotoxicity, Pad-dry cure method, *Chromolaena odorata*

I. INTRODUCTION

Textiles have been used since ancient times as suitable covering material on wounds to prevent infection, for absorbing exudates and to accelerate healing. The primary function of wound dressing is to avoid penetration and protect the wound site from infection. Historically, plants, honey pastes, and animal fats were used as wound healing materials. Traditional dressing materials consist of absorptive layer made of cotton or rayon gauze [Eriksson and Sandsjo, 2015]. Novel wound dressings containing antimicrobial agents have paved a way to manage wound infection. A wound is defined as a disruption in the continuity of the epithelial lining of the skin or mucosa resulting from physical or thermal damage. According to the duration and nature of healing process, the wound is categorized as acute and chronic [Robson *et al.*, 2001, Szycher & Lee 1992]. An acute wound is an injury to the skin that occurs suddenly due to accident or surgical injury. It heals at a predictable and expected time frame usually within 8-12 weeks depending on the size, depth and the extent of damage in the epidermis and dermis layer of the skin [Schreml 2010, Rajendran & Anand 2011]. Chronic wounds generally result from decubitus ulcer, leg ulcer and burns. Wound healing is a dynamic and complex process of tissue regeneration and growth progress through four different phases (i) the coagulation and haemostasis phase (immediately after injury); (ii) the inflammatory phase, (shortly after injury to tissue) during which swelling takes place; (iii) the proliferation period, where new tissues and blood vessels are formed and (iv) the maturation phase, in which remodeling of new tissues takes place [Dowsett & Newton 2005, Hunt *et al.*, 2000]. These phases occur in an ordered manner overlapping with each other in a well-connected cascade [Rivera & Spencer 2007, Strecker-McGraw *et al.*, 2007]. Promotion of these phases are largely depending on the wound type and its associated pathological conditions and the type of dressing material. With the advancement in technology, currently, different types of wound dressing materials are available for all types of wounds. But the selection of a material for a particular wound is important to achieve faster healing [Baxter 2015].

The study and commercialization of natural antimicrobial sources have been largely limited to certain plants. However, commercial wound dressing applications with such natural products have not yet been reported, except for chitosan, where commercial applications with silver and zinc oxide antimicrobial treatment are available. Hence, more research needs to be focused on understanding the mechanism of drug resistance, exploring novelty and safer use of antimicrobial sources along

with controlled use of antibiotics for comparison. Therefore, the current study is aimed to explore and develop wound dressing bandage with antimicrobial activity using the extract obtained from herbal plant to achieve faster healing.

II. MATERIALS AND METHODS

2.1. Plant Materials

The fresh leaves obtained from the herbal plant (*Chromolaena odorata*) was collected, washed thoroughly using tap water and air dried under shade for 3 days. After the complete drying of leaves, it was powdered and used for the experimental procedures (Fig. 1).



Fig.1 a) *Chromolaena odorata*



b) *C. odorata* powdered leaves

2.2 Preparation of wound dressing bandage

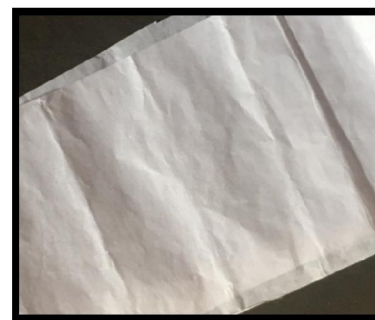
The wound dressing bandage was prepared in the form of three layers (primary, wound contact, peel off) as shown in (Fig. 2).



a) Primary Layer



b) Antimicrobial Coated Viscose fabric



c) Peel off layer

Figure 2: Development of wound dressing bandage

A. Primary layer – The first layer that comes in contact with skin around the wound is a 3M microporous tape. The tape is also coated with acrylic adhesive and is not irritant to skin.

B. Wound contact layer – The second layer, which is the wound contact layer is loaded with antimicrobial drug obtained from the leaf extract of plant. The material used for finishing is made by of non- woven viscose fabric.

C. Peel off layer - The peel layer of bandage (third layer), is made up of cellulose-based non-sticky paper, which is commercially available.

2.3. Extraction of Plant Materials

30 g of leaf powder obtained from the herbal plant was extracted using 250 ml of 95% ethanol in Soxhlet apparatus for 8h. Now, ethanol was subjected for evaporation with the method of reflex extraction [Zhang *et.al.*, 2018]. After evaporation the extract was taken out and kept at room temperature to evaporate traces of ethanol if present. The obtained crude extract was stored in sterile centrifuge tube at 4°C until further use. The aqueous extraction of the plant leaf was carried out using 20g of the powdered sample in 100 ml of distilled water, kept in a mechanical shaker for 24 h and then centrifuged at 5000 rpm for 5 min and filtered through Whatman No. 1 filter paper.

2.4. Phytochemical Screening

The phytochemical screening [Matawali *et.al.*, 2016] was carried out by soaking the crude extract of leaf in 20 ml of DMSO until the powder became fully saturated in 100 ml conical flask.

2.5. Antimicrobial Activity of Plant Extract

A. Preparation of Bacterial Inoculum

The bacterial strains of *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumonia* (ATCC 4352), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 15442) was used in order to study the antibacterial activity of plant extract obtained from *Chromolaena odorata*. The bacterial strains were inoculated in nutrient broth and kept for overnight incubation at 37°C. The cultures were observed for turbidity and the bacterial inoculum concentration was adjusted to 10⁸ CFU/ml. The final concentration of the bacterial inoculum was adjusted to 10⁵ CFU/ml by adding 5 µl of 10⁸ CFU/ml to 5 ml of KH₂PO₄ buffer solution and considered to be 10⁵ CFU/ml.

B. Antimicrobial Well Diffusion Method

Antibacterial activity of the plant extract was carried out using well diffusion method. 2 ml of crude plant extract was diluted in 8 ml of 1% DMSO solution. 100 µl of the bacterial inoculums containing 10⁵ CFU/ml was spread over the plates containing Muller Hinton Agar. Now, 0.8cm wells were cut in the agar plates, 100 µl of plant extract was added to the wells and kept for incubation at 37 ° C for 18-24 h. The zone of inhibition was calculated for the antibacterial activity of the plant extract.

2.6. Minimum Inhibition Concentration (MIC)

To determine minimum inhibition concentration, 2 ml of plant extract was dissolved in 8 ml of DMSO solution. The 24 hours subculture of the test organism *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 4352), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442) was adjusted to 10⁵ dilutions and determined for MIC study. The different dilutions were taken for MIC. The dilution was added to each tube of the plant extract from the prepared stock solution ranging from 50 µl to 500 µl. The concentration of MIC was marked according to the volume of plant extract being added. The volume of nutrient broth ranged from 0 to 1000 µl. The volume of bacterial culture added remained constant at 10 µl. A separate test tube containing only nutrient broth was made as control. Now all the tubes containing test culture and control sample was incubated at 37 ° C for 24 h. After the incubation period, MIC was determined based on the occurrence of turbidity.

2.7. Analysis of Antimicrobial finished fabric

A. Pad- dry cure method

MLR – 1:40 Antimicrobial stock solution: as per MIC concentration (400 µl) Cross-linking agent (citric acid) – 6%

The fabric was immersed in antimicrobial stock solution containing cross linking agent for 10 min and passed through pneumatic padding mangle at a speed of 3m/min with the pressure of 1 Kg/ cm² in order to remove excess solution, ensuring the wet pick up of 70%. Finally, the fabric was dried in shade and cured for 3 minutes at 140°C.

2.8. Physical Parameter analysis for Herbal Coated Fabric

A. pH of the fabric (Standard used: ISO 3071)

The pH of the aqueous extract of the herbal coated fabric was measured. Initially, non-coated fabric was dipped in 100ml of distilled water, taken as control and its pH was recorded. Then pH was determined for the coated sample fabric immersed in 100ml of distilled water.

B. Absorbency of the fabric (Standard used: EN13726)

The wound dressings were sectioned into 5X5 cm samples, weighed up to 1g and incubated in artificial exudates (saline and non-infected exudates) at 37°C. The free swell absorbency following 30 min of incubation was calculated by measuring the initial and final weight, before and after the incubation. The non-coated fabric immersed and incubated in normal distilled water was considered to be the control. Finally, the absorbency was calculated as g/g in the form of triplicates.

2.9. Analysis of Biological parameters of the coated wound dressing fabric

A. Antibacterial activity of Textile Materials (International Standard AATCC 100-2004)

The concentration of the test microorganism (*Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 4352) was standardized to 10⁵ CFU/ml. The sample fabric was cut into circular swatches with a diameter of 4.8 cm ± 0.5 cm and weighed for 1g.

B. Cytotoxicity

The direct contact method [MTT assay- ISO 10993-5] used to analyze the cytotoxicity of finalized herbal sample. A known amount of cell suspension (1 x 10⁴ cells/ml of L929 mouse fibroblast, purchased from NCCS, Pune) was pipetted and the cells were evenly distributed onto 96 well. The culture was incubated at (37±1) °C in CO₂ incubator, the morphology and sub confluence of the cells were examined with the help of inverted phase contrast microscope. Fresh Minimum Essential Media (MEM) culture medium (10%) was added to each vessel and fabric samples were placed on the middle of each vessel over the cell layer ensuring that the specimen covers 1/10th of the cell surface layer. Replicate vessels were prepared for the negative, positive, and fabric samples. Now, the vessels were incubated at (37±1)°C in CO₂ incubator for 24 hours. After that the cultural medium was discarded. The percentage of inhibition of cell growth was calculated for positive control, negative control, and plant extract treated fabric (International standard ISO10993-5:2009(E), 2009).

$$\text{cytotoxicity index} = 100 \times (C - T) \div C$$

Where, C=Control, T= Treated sample

C. In Vitro Scratch Wound Assay

The fibroblast L929 cells were seeded in a 24-well cell culture plate. A linear scratch was made on a confluent cell monolayer using 200µl pipette tip. Cell debris was washed using Dulbecco's Modified Eagle's (DME) medium. After the addition of herbal coated fabric, images were captured periodically at (0, 4, 18 and 24thhr) using Nikon Eclipse TS100 inverted microscope. A control was also tested for the comparative study based on the percentage of wound healing. The cellular gap in the cell monolayer was measured for the control and sample for the respective time intervals (0, 4, 18 & 24 hrs).

2.10 Designing and Development of antimicrobial wound dressing bandage

The development of wound dressing consists of three layers; top layer (3M microporous tape) with a dimension of 6X5 cm which adheres to the skin, second layer (100% spun viscose fabric of lace, 3X3 cm) medicated wound contact layer treated with *C. odorata* extracts, which can absorb the exudates. The third layer or the peel off layer (non-sticky cellulose paper, dimension 7X7 cm) was used to protect the sticky adhesive of the tape and to make it convenient for the easy application of bandage.

III. RESULTS

3.1. Phytochemical Screening of *Chromolaena odorata* Leaf Extracts

The phytochemical screening of the ethanolic leaf extract of *Chromolaena odorata* showed the presence of compounds like Tannins, saponins, alkaloids, flavonoids, terpenoids, phenols, coumarins and the absence of glycosides during the preliminary screening of phytochemical analysis (**Table 1**).

Table 1: Phytochemical screening of *Chromolaena odorata* leaf extract

PHYTO CONSTITUENTS	PRESENCE/ ABSENCE
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Phenolic compounds	+
Glycosides	-
Coumarins	+

(+) present, (-) absent

3.2. Assessment of Anti-Microbial Activity (Disk Diffusion Method)

The antimicrobial activity with the ethanol and aqueous leaf extracts of *Chromolaena odorata* was determined using agar well diffusion method (**Fig.3**).

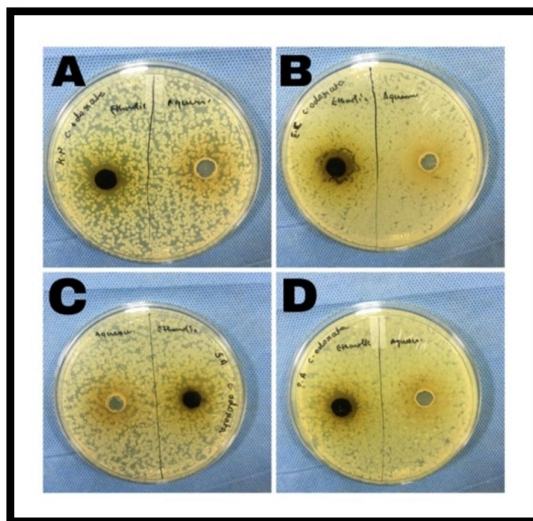


Figure 3: Testing the antimicrobial efficiency of plant extract

1. *Klebsiella pneumoniae* (ATCC 4352)- Ethanolic & Aqueous Extract
2. *Escherichia coli* (ATCC 25922) - Ethanolic & Aqueous Extract
3. *Staphylococcus aureus* (ATCC 6538) - Ethanolic & Aqueous Extract
4. *Pseudomonas aeruginosa* (ATCC 15442) - Ethanolic & Aqueous Extract

The antibacterial activity was measured on Muller-Hinton agar plates as zone of inhibition around the wells in which the extract solution was placed. The ethanolic extract produced measurable amounts (1mg/ml) of antibacterial activity on bacteria *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 4352 and *Pseudomonas aeruginosa* ATCC 15442, when compared to the aqueous solution extract as shown in **Table.2**. It was found that aqueous plant extract showed no relevant zone of inhibition. The zone of inhibition was compared with the standard chloramphenicol (1mg / ml).

Table 2: Zone of inhibition of ethanolic and aqueous extracts of *C. odorata*

Sl. No	Bacterial Strain	Standard reference	Zone of Inhibition (in cm)		Zone of inhibition (in mm)		Activity Strength	
		Chloramphenicol (zone of inhibition in mm)	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract
1	<i>Pseudomonas aeruginosa</i> ATCC 15442	27±2	1.7 cm	-	17mm	-	Strong activity	No activity
2	<i>Escherichia coli</i> ATCC 25922	24±2	1.5 cm	-	15mm	-	Strong activity	No activity
3	<i>Klebsiella pneumoniae</i> ATCC 4352	30±2	1.9 cm	-	19mm	-	Strong activity	No activity
4	<i>Staphylococcus aureus</i> ATCC 6538	24±2	1.2 cm	-	12mm	-	Medium activity	No activity

Note -Inhibition Strength Indicator:

Diameter of inhibition zone ≥ 15 mm: Strong (sensitive)

Diameter of inhibition zone 10 mm -14.5 mm: Medium (sensitive)

Diameter of inhibition zone ≤ 9 mm: Weak (Resistant)

Diameter of inhibition 0 mm: No activity (resistant)

3.3. Minimum Inhibitory Concentration

The minimum inhibitory concentration of the plant extract was calculated by checking the turbidity obtained as a result of microbial growth from each of the dilution tubes. The dilution of the plant extract used was in the range from 50 μ l to 500 μ l. The results were obtained after incubation and it was found that the concentration of 400 μ l is the MIC for *C.odorata* plant extract tested against the four bacterial strains shown in **Table.3**.

Table 3: Minimum inhibitory concentration values.

Bacterial Strain	Concentration of plant extract (μ l)									
	0	50	100	200	250	300	350	400	450	500
<i>Pseudomonas aeruginosa</i> ATCC 15442	+	+	+	+	+	+	+	-	-	-
<i>Escherichia coli</i> ATCC 25922	+	+	+	+	+	+	+	-	-	-
<i>Klebsiella pneumonia</i> ATCC 4352	+	+	+	+	+	+	+	-	-	-
<i>Staphylococcus aureus</i> ATCC 6538	+	+	+	+	+	+	+	-	-	-

(+) indicates presence of growth, (-) indicates the absence of growth

3.4. Analysis of Physical parameters of the Antimicrobial finished fabric

A. Analysis of pH for the fabric

The pH of antimicrobial finished fabric was evaluated in order to determine, whether the fabric provides favorable pH environment for the process of effective wound healing. The pH of herbal finished fabric was found to be 2.94, which is considered ideal for wound healing. A non-coated sample was used as a control, providing a neutral pH of 7.64. The pH of the sample is reported at the given temperature of 25 $^{\circ}$ C (**Table.4**).

Table 4: Recorded pH value of the sample with control

Sl. No	Fabric	Recorded pH value at 25°C
1	Sample (Antimicrobial finished fabric)	2.94
2	Control (non coated fabric)	7.64

3.4.2 Analysis of Absorbency for the Antimicrobial Finished Fabric

The results for the absorbency of wound dressing in saline (simulated exudates) in the antimicrobial finished fabric showed greater absorbency (8.04 g/g). The absorbency of non-infected wound exudates was also observed along with control. In the case of non-infected exudates, the absorbency of antimicrobial finished fabric showed greater absorbency (8.59 g/g) (Table 5)

Table 5: Absorbency of fabric in saline and non-infected exudates

	Replicates	Initial Weight	Final Weight	Added Value	Absorbency (G/G)	Absorbency (%)
Saline	R1	1.1	9.83	8.73	7.93	79.3
	R2	1.02	8.75	7.73	7.57	75.7
	R3	1.00	9.61	8.61	8.61	86.1
Average	-	-	-	-	8.04	80.4
Non-Infected wound exudates	R1	1.02	9.85	8.83	8.65	86.5
	R2	1.01	9.64	8.63	8.54	85.4
	R3	1.00	9.57	8.57	8.57	85.7
Average	-	-	-	-	8.59	85.9

(G/G) is defined as the swollen weight of SAP (g) divided by the dried weight of SAP (g).

3.5. Analysis of Biological parameters for the coated wound dressing fabric

A. Assessment of Antibacterial Finishes on Textile Materials (AATCC 100-2004)

The bacterial reduction for the fabric treated with *C. odorata* plant extract is provided in Table 6. The bacterial colonies of *S. aureus* and *K. pneumonia* for 0th and 24th hrs of the first three dilutions (10⁰, 10¹ and 10²) in replicate plates was considered. The quantitative analysis of bacterial reduction in the herbal finished fabric by AATCC 100-2004 test method showed that the sample exhibited maximum bacterial reduction of 99.6% against *S. aureus*, when compared with *K. pneumoniae* of 75.09%. It is found that finished fabric with *Chromolaena odorata* leaf extract exhibited maximum reduction tested against the bacterial colonies.

Table 6: Percentage reduction of bacterial colonies

Bacterial Strain	Percentage reduction (%)	
	0 hour	24 th hour
<i>Staphylococcus aureus</i> ATCC 6538	20	99.6
<i>Klebsiella pneumonia</i> ATCC 4352	27.7	75.09

B. Cytotoxicity (MTT assay- ISO 10993-5)

The cytotoxicity of antimicrobial coated fabric was tested and the cell viability percentage is summarized as shown in Table 7

Table 7: Cytotoxicity percentage of the fabric treated with *C. odorata* extract

Source	Cytotoxicity (%)	Cell viability (%)	Cytotoxicity Reactivity
<i>C. odorata</i> treated fabric	43	57	Mild

As per ISO 10993-5 standard the test sample containing antimicrobial coated viscose fabric showed mild toxicity to L929 cells after 24hr contact. The control did not show any of the cytotoxicity reactivity (Fig. 4.). It is clear from It is found that the test sample is having mild toxicity of 43% and cell viability of 57%. As per the standard test method (ISO 10993-5), The biological evaluation of medical devices for vitro cytotoxicity cell viability testing shows that reduction in more than 30% cells is considered to have cytotoxicity effect (ISO 10993-5, 2009, Association for the Advancement of Medical Instrumentation) **Table.8.**

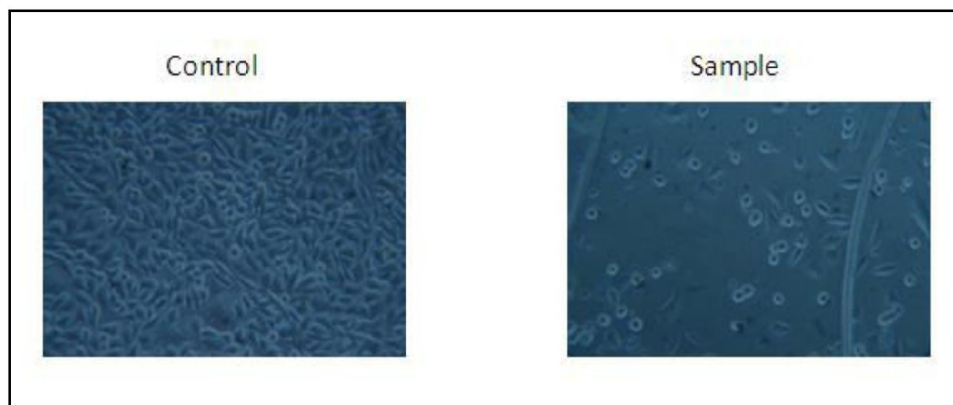


Figure 4: Cytotoxicity testing images of the control and sample

Table 8: Evaluation Criteria for cytotoxicity

Sl. No	Grade (%)	Reactivity
1	0	None
2	1-20	Slight
3	21-50	Mild
4	51-70	Moderate
5	>71	Severe

C. Scratch Wound Assay

The healing rate of antimicrobial coated viscose fabric was found to be 75% and complete healing was observed after 24 hrs. The results were compared with control and the healing percentage of the control sample is shown in (Fig. 5).

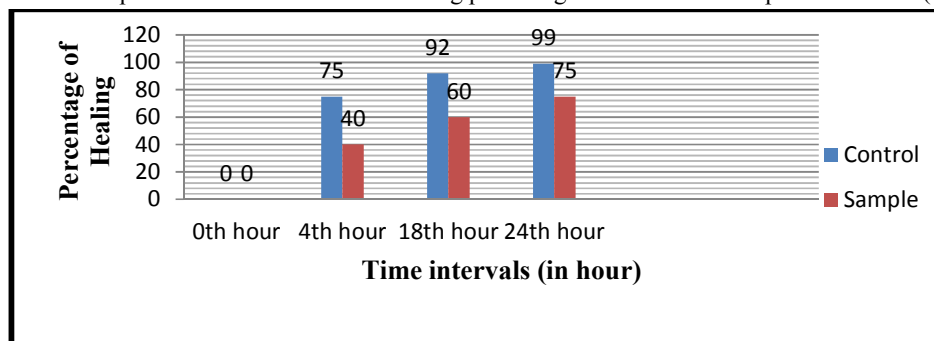


Figure 5: Comparative study of wound healing percentage for control and sample

3.6. Designing and development of antimicrobial wound dressing bandage

The wound dressing consisted of top layer which is made up of 3M Microporous tape with acrylic adhesive and aids as a scaffold for the attachment of cells and their proliferation. The wound contact layer absorbs the exudates and it contains the antimicrobial finish. Antimicrobial analysis of non-woven viscose spun lace showed that the fabric treated with *C. odorata* extracts have significant antimicrobial activity. The prepared wound dressing materials and bandages were stored in zip-lock covers until further use (Fig.6). The designing of the developed wound dressing materials is shown in **Table. 9.**



Figure 6: Wound Dressing Bio-bandage

Table 9: Details of the developed wound dressing

Layer	Composition	Function	Dimension
Top layer	3M Microporous tape with acrylic adhesive	Adhesion to the skin	6x5cm
Wound contact layer/ medicated layer	Antimicrobial finished spun lace non-woven viscose fabric	Wound healing, inhibits bacterial infection, absorption of exudates	3x3 cm
Peel off layer	Cellulose based non sticky paper	Ease for application	7x7 cm

The end product is an antimicrobial wound dressing bandage of size 6X6 cm based on a natural wound healer, *Chromolaena odorata* leaf extracts which can be used for superficial dermal wounds.

V. DISCUSSION

From the Phytochemical screening results **Fig.1 & Table.1** represents Alkaloid's phytochemicals possess antibacterial activity as well as antibiotic enhancing activity against all bacterial strains [Cushnie *et.al.*, 2014]. Tannins are water-soluble polyphenols which possess astringent property and make complex with enzymes. Several microbial enzymes in purified forms when mixed with tannins possess zone of inhibition [Akiyama, *et.al.*, 2001]. Flavonoids are active antibacterial constituents against an extensive range of microbes which have the ability to make complex structure with bacterial cell walls [Cowan, 1999]. Steroids exhibit antibacterial activity precisely allied with membrane lipids [Epanand, *et.al.*, 2007]. Saponins are complex group of high molecular weight compounds which possess both antimicrobial and antioxidants properties to mitigate the growth of bacteria [Akinpelu, *et.al.*, 2007].

Table.2 & Fig.3 represents the quantitative analysis of zone of inhibition for samples padded with the ethanol and aqueous leaf extracts of *Chromolaena odorata* was determined using agar well diffusion method. Compared to the aqueous and ethanolic extract of the leaves, the ethanolic extract produced measurable amounts of antibacterial activity on bacteria such as *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 15442). The zone of inhibition ranged from 12mm to 17mm which is considered to have a strong sensitivity for the plant extracts. The results were compared with the standard reference of antibiotic Chloramphenicol. The Minimum inhibitory concentration of the plant extract to inhibit the growth of the bacteria is also calculated to know the least concentration to be loaded in the wound dressing. **Table.3, 4, 5 & 6** results show that the volume of 400µl can inhibit the growth of the bacteria. The inhibitory action of the alkaloid possesses antibacterial activity against all bacterial strains [Gurrapu, & Mamidala, 2017].

In **Fig.2** the development of wound dressing bandage represents Micropore layer of primary layer consists of a conformable, non- extensible nonwoven fabric manufactured from 100% viscose, coated with a layer of acrylic adhesive. The tape can be easily torn to desired length. When applied on to the skin, micropore can withstand little exposure to water without losing its adhesive nature. The tape is permeable for both water and water vapor, permits the passage of sweat and secretions from the surface of body into the environment and preventing the maceration of skin. It has higher porosity and larger surface area as to provide open structure for the drainage of exudates and reduces that may risk the outcome of secondary infection. The absence of dust and ease of processing non-woven fabric serves to be excellent material for the application of wound dressing [Vloemans *et al.*, 2001]. The porous structure of fabric allows the skin to breathe, which reduces the risk of wound

infection. The soft, light, and elastic nature of bandage makes it easier to put even where there is body movement. The high absorption capability along with safety features and comfort, makes the fabric user friendly. The fabric also has inherent biodegradability, which is an additional benefit for the development of bandage.

Fig.4 & Table.7 & 8 Cytotoxicity testing images of the control and sample fabric chosen in Non woven Viscose and the finish is done by Pad- dry cure method. The Physical and biological parameters of the antimicrobial finished fabric is assessed by various standards. The physical parameters such as pH and absorbency which is very important for a wound dressing is been analyzed for the antimicrobial finished fabric. The results show that the fabric has optimum pH of 2.96 at 25°C and an absorbency of 85.9 % in non infected wound exudates. This result concludes that the particular fabric is suitable for use as a wound dressing. Biological parameters such as antimicrobial assessment (AATCC 100-2004), cytotoxicity, and scratch wound assay are also analyzed for understanding the efficacy of the fabric. **Fig.5** graphical representation of the comparative study of wound healing percentage for control and sample summarize that the fabric can leach out the antimicrobial drug, it also shows a great reduction percentage in the number of bacterial colonies. This finding implies in **Fig.6 & Table.9** shows that when fabric is used as a wound dressing, it can leach the antimicrobial drug, which ultimately inhibits the growth of bacteria in the wound. The cytotoxicity result says that the fabric is of mild toxicity, but can still be used for wound dressing. The in vitro Scratch wound assay result gives us an idea of the potential of wound healing of the fabric, which is also at an optimum level. Textiles intended to be used as medical textiles that have indirect or direct contact with human tissues must be assessed for biocompatibility before they are introduced into the market. Unless the product meets the strict standardized protocol, it cannot be used in humans. When a product is to be used in a human body, strict criteria of biocompatibility, nontoxic, non - carcinogenic, non-mutagenic, non-thrombotic, non-hemolytic must be strictly enforced [**Prabu and Ravi, 2012**].

VI. CONCLUSION

The experiment was carried out for the development of antimicrobial wound dressing bandages from the extracted leaf extracts of *Chromolaena odorata*. This herbal plant is an invasive weed, which has the potential for wound healing activities. The potential of this plant has been explored for the development of wound dressing bandages.

The phytochemical investigation exhibits the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids and coumarins etc. These compounds exhibit vital roles for antibacterial effect. Compared to the aqueous and ethanolic extract of the leaves, the ethanolic extract produced measurable amounts of antibacterial activity on Gram Positive and Gram-Negative bacteria. The zone of inhibition ranged from 12mm to 17mm which is considered to have a strong sensitivity for the plant extracts.

After obtaining the MIC values of the plant extract, the antimicrobial drug is been coated on the fabric. The fabric chosen in Non woven Viscose and the finish is done by Pad- dry cure method. The Physical and biological parameters of the antimicrobial finished fabric is assessed by various standards. The results show that the fabric has optimum pH of 2.96 at 25°C and an absorbency of 85.9 % in non infected wound exudates. This result concludes that the particular fabric is suitable for use as a wound dressing. Biological parameters such as antimicrobial assessment, cytotoxicity, and scratch wound assay are also analyzed for understanding the efficacy of the fabric. The result of AATCC 100-2004 summarize that the fabric can leach out the antimicrobial drug, it also shows a great reduction percentage in the number of bacterial colonies. This finding implies that when fabric is used as a wound dressing, it can leach the antimicrobial drug, which ultimately inhibits the growth of bacteria in the wound. The in vitro Scratch wound assay result gives us an idea of the potential of wound healing of the fabric, which is also at an optimum level. The end product is an antimicrobial wound dressing bandage of size 6X6 cm based on a natural wound healer, *Chromolaena odorata* leaf extracts which can be used for superficial dermal wounds. Considering all the facts, these herbal finished bandages can be implemented as medical bandages in commercial basis.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Rathi, Department of Microbiology, Centre of Excellence for Medical Textiles Biotechnology Laboratory, Coimbatore, India, for providing the basic laboratory facility to undergo this research. Also, special thanks Nehru Arts and Science College, Coimbatore, Tamil Nadu, India for supporting this research with fund.

REFERENCES

- [1]. AATCC 100: (2004)., Antibacterial Finishes on Textile Materials: Assessment of developed from the American Association of Textile Chemists and Colorists.
- [2]. AOAC: (2005), Official methods of analysis, 18th edn., Association of Official Analytical Chemists, Washington DC, USA.
- [3]. Matawali. A., Chin L.P., Eng H.S., Gansau J.A. (2016). Antibacterial and phytochemical investigations of *Mikania micrantha* HBK (Asteraceae) from Sabah, Malaysia. *Trans. Sci. Technol.* 3(1–2),244–250.
- [4]. Akinpelu B.A., Igbeneghu O.A., Awotunde A.I., Iwalewa E.O., Oyedapo E.O.O.O., (2014). Antioxidant and antibacterial activities of saponin fractions of *Erythropheleum suaveolens* (Guill. and Perri.) stem bark extract. *Sci. Res. Essays.* 9(18), 826–833.
- [5]. Baxter E. (2015). Complete crime scene investigation handbook: CRC press.,31–3.
- [6]. Dowsett C, Newton H. (2005). Wound bed preparation: TIME in practice. *WOUNDS UK.*; 1:58–70.
- [7]. Eriksson and Sandsjo (2015). Three-dimensional Fabrics as Medical Textiles, *Advances in 3D Textiles*, Publisher: Wood head Publishing, Chapter: 12:305-340.
- [8]. Akiyama H., Fujii K., Yamasaki O., Oono T., Iwatsuki K., (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. *J. Antimicrob. Chemother.*, 48(4), 487–491.
- [9]. Hunt TK, Hopf H, Hussain Z. (2000). Physiology of wound healing. *Adv Skin Wound Care*; 13:6–11.
- [10]. ISO 3071:(2005(E)). Textiles — Determination of pH of aqueous extracts.
- [11]. Cowan M.M., (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12(4), 564–582.
- [12]. Prabhu, V., Ravi, S., (2012). Isolation of a novel triterpene from the essential oil of fresh leaves of *Chromolaena odorata* and its *in vitro* cytotoxic activity against HepG2 cancer cell line. *Journal of Applied Pharmaceutical Science* 2:132-136
- [13]. Epan R.F., Savage P.B., Epan R.M., (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). *Biochim. Biophys. Acta. Biomembr.*, 1768(10), 2500–2509.
- [14]. Rajendran S, Anand SC. (2011) Hi-tech textiles for interactive wound therapies: *Handbook of Medical Textiles*.
- [15]. Rivera AE, Spencer JM. (2007). Clinical aspects of full-thickness wound healing. *Clin Dermatol.* 25:39–48.
- [16]. Robson MC, Steed DL, Franz MG. (2001). Wound healing: biological features and approaches to maximize healing trajectories. *Curr Prob Surg.* 38:77–89.
- [17]. Gurrapu S., Mamidala E., (2017). In vitro antibacterial activity of alkaloids isolated from leaves of *Eclipta alba* against human pathogenic bacteria. *Pharmacogn. J.*, 9(4), 573–577.
- [18]. Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas P. (2010). Oxygen in acute and chronic wound healing. *Br J Dermatol.* 163:257–68.
- [19]. Strecker-McGraw MK, Jones TR, Baer DG.(2007). Soft tissue wounds and principles of healing. *Emerg Med Clin North Am.* 25:1–22.
- [20]. Szycher M, Lee SJ. (1992) Modern wound dressings: a systemic approach to wound healing. *J Biomater Appl.* 7:142–213.
- [21]. Cushnie T.T., Cushnie B., Lamb A.J., (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int. J. Antimicrob. Agents.* 44(5), 377–386.
- [22]. Vloemans AF, Soesman AM, Kreis RW, and Middelkoop E: (2001). A newly developed hydrofibre dressing in the treatment of partial-thickness burns. *Burns.* 27: 167.
- [23]. Zhang W.Q., Lin G.L. and Ye C.W. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine.* 13(20),1-26.