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Development of medical textile product using chitosan incorporated herbal extract (*Aristolochia bracteolate)*

M. Sumithra
Department of Textiles and Apparel Design, Bharathiar University, Coimbatore, (TN) - India

Abstract

In the current research, biopolymer herbal composites have been prepared using Chitosan and herbal extract of *Aristolochia bracteolate*. It has wide range of application in the field of pharmaceutical industries. Chitosan was isolated from exoskeleton of crab's shell by means of demineralization and deacetylation process. Purification of chitosan was achieved by NaOH and acetic acid. The herbal extract was prepared by 80% methanol. The composite was prepared by mixing the biopolymer and herbal extract in a magnetic stirrer for 20 minutes. The prepared biopolymer and the herbal extract composite were tested for its wound healing activity by using In vitro Wound Scratch Assay. The wound healing study was performed in fibroblast cell lines at various concentrations. The Chitosan incorporated herbal extract showed 80% wound healing activity in 75µL at 18 hrs time period. The selected concentration was used for finishing the prepared biopolymer composite to the cotton woven fabrics. The finished fabric was kept as a medicated area and were developed in to a medical product i.e. Band-Aid.

Keywords: *Aristolochiabracteolate*,bandages,,crab shell, chitin and chitosan purification

Introduction

Medical textiles also known as Healthcare Textiles.Advanced medical textiles are significantly developing area because of their major expansion in such fields like wound healing and controlled release, bandaging, implantable devices as well as medical devices and development of new intelligent textile products. Medical Textiles is one of the most rapidly expanding sectors in the technical textilemarket. It is one of the major growth areas within technical textiles and the use of textile materials for medical and healthcare products ranges from simple gauze or bandage materials to scaffolds for tissue culturing and a large variety for permanent body implants. Textile products are present in the field of human hygiene and medical practice. Their use is based on a number of typical basic textile properties like softness, lightness, flexibility, absorption and filtering etc [1] .Chitosan derived from acetylating of chitin, the marine polymer was a best alternative for heavy metals in finishing the medical fabrics. Chitosan is a natural, nontoxic,Chitosan is considered as regenerating raw material. It is the most abundant natural polymer after the cellulose.

*** Corresponding Author**
E.mail: mithrasumi6@rediffmail.com

The natural occurrence amounts to more than 1000 tons per year, of it been allotted approx. 70% from the bowls of sea animals [2].*Aristolochia* is a large plant genus with over 500 species,belongs to the family *Aristolochiacea*e . In the indigenous system of medicine, the plant was used for the treatment of skin diseases, inflammation and purgative. Root extract was accounted to have anti-bacterial activity.*Aristolochia* species has been used extensively in the traditional[3].*Aristolochia bracteolate* is an herbaceous perennial medicinal plant with cordate leaves and dark purple colour tubular flowers belonging to the family *Aristolochiaceae*. The plant commonly called as Worm killer in English and Aadutheendapalai in Tamil, due to supposed anthelminthic activity and trypanocidal effect [4].The objectives of this study are to produce cotton woven fabrics,To produce chitosen incorporated herbal extract (*Aristolochiabracteolata*) for finishing the cotton fabric, To produce bandages using crab cells,To impart the extract of the herbs using spray method and To evaluate the wound healing property.

Material and Methods

Purchase of raw materials

The crab shells were purchased from Ukkadam fish market, Coimbatore, Tamilnadu, India. The chemicals used for the study was purchased from, The Precision Scientific Company, Coimbatore, Tamilnadu, India.

Selection of natural herbs

Herbs were purchased from Paripoorna herbal store, Coimbatore, Tamilnadu, India.

steps For herbal extraction & sample preparation

Step 1: Synthesis of Chitosan nanoparticles Samplepreparation(Abdou *et al.*, 2008)

The crab purchased was processed in laboratory condition to separate exoskeletons form flesh. The crab exoskeletons collected were placed in Ziploc bags and refrigerated overnight. Moisture content was determined on the crab waste by first crushing exoskeletons into smaller pieces using a meat tenderizer. Approximately 10grams of crushed crab's exoskeletons wet samples were placed on foil paper and measured using a Mettle balance. There were five measurements made of the wet crushed crab exoskeletons samples. The samples were then labeled and oven-dried for 4 consecutive days at 65˚C until constant weight.

Extraction Of Chitin And Chitosan (Abdou e*t Al.*, 2008**)**

The crab's exoskeletons were placed in 250ml beakers and treated in boiling sodium hydroxide (2% and 4% v/v) for 1hour in order to dissolve the proteins and sugars thus isolating the crude chitin. After the samples were boiled in the sodium hydroxide, the beakers containing the crab shell samples were removed from the hot plate, allowed cooling for 30minutes at room temperature. The exoskeletons were then further crushed to pieces of 0.5-5.0mm using a mortar and pestle.

Demineralization (abdou*et al.*, 2008**)**

The grounded exoskeletons of crab samples weighing approximately 25g each. Each sub-sample was demineralized with 100ml of HCl using concentrations 0.5% concentration. The remaining chitin was washed with deionized water, which was then drained off. The chitin was further converted into chitosan by the process of deacetylation. To soak for 24hours to remove the minerals (mainly calcium) then treated for 1hour with 50ml of a 2% NaOH.

Deacetylation (Abdou*et al.*, 2008**)**

The deacetylation process was carried out by adding 100ml of 50% NaOH then boiled at 100˚C for 2hours on a hot plate. The samples were then placed under the hood and cooled for 30minutes at room temperature. Afterwards the samples were washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The prepared chitosan was then placed in 250ml beakers and labeled according to the treatment used. The samples were then left uncovered and oven dried at 120˚C for 24hours. The chitosan was then in a creamy-white form.

Chitosan Purification Procedure

The crude chitosan dissolved in 2% W/V acetic acid. Then the insoluble material is removed given a clear supernatant solution, which is neutralized with NaOH result in a purified sample of chitosan as a white precipitate. Further purification may be necessary to prepare medical and pharmaceutical grade chitosan (50%).

crab shell chitin chitosan purification

Chitosan Powder

Step 2: Herbal extract (Methanol)

Solvent extraction of collected herbs

Extraction was carried out by dissolving 12grams of the Selected herbal powder (*Aristolochiabracteolata*) in 200ml of 80% methanol, kept overnight under shaking condition. Then the extract was filtered using

Whatmann no.1 filter paper, filtrate was collected and collected and stored for further studies.

Preparation of composite sample

5 grams of obtained chitosan was added in 50 ml of distilled water. 50 ml of herbal extract was added to prepared chitosan and by keeping mix well and keep it in industrial magnetic starrier for 20 mins at 200 rpm then after finishing on fabric.

Step 3: In vitro Wound Scratch Assay

Procedure

Fibroblast (L929) cells were grown in 24 well plates at a density of 1.00 x10^5 cells/ml and cultured until ~ 80 % confluency. A small linear scratch was created in the confluent monolayer by gently scraping with sterile cell scrapper as per the method described by Liang et al. (2007). Cells were thoroughly rinsed with 1 X PBS to remove cellular debris and treated with different concentration (25 µl). Cell proliferation was monitored at different time points: 1 h, 4 h, and 18 h and images of the migrated cells were taken at all different time points using digital camera (Nikon, Tokyo, Japan) connected to the inverted phase contrast microscope (Radical instruments, India). Extent of wound healing was determined by the distance traversed by cells migrating into the denuded area.

Finishing on Fabric

Step 4: Finishing on to fabric (Cotton woven knitted)

The formal structure of a woven fabric is defined by weave, **thread density**, crimp and **yarn count**. Woven fabrics are made by using two or more sets of yarn interlaced at right angles to each other. Much variety is produced by weaving. Woven fabrics are generally more durable. A woven fabric which isused inclothing and garmentsor for decoration and covering purposes. Without these application woven fabric also used as**sportswear**, medical applications, **textiles for electronics** and air bag construction in automotive engineering.

The importance of woven fabrics increases constantly. Starting from traditional uses mainly in clothing applications, woven fabrics today are key materials for structural, electronic, telecommunications, medical, aerospace and other technical application fields. The new application fields of the woven fabrics is directly reflected in the contents of the book. A selected collection of papers in the technological state-of-the-art builds the book "Advances in Modern Woven Fabrics Technology". It is written by internationally recognized specialists and pioneers of the particular fields. The chapters embrace technological areas with major importance, while maintaining a high scientific level. This interdisciplinary book will be useful for the textile family member as well as for the experts of the related engineering fields. The open access character of the book will allow a worldwide and direct access to its contents, supporting the members of the academic and industrial community.

The extraction was finished on to medicated fabric by the following method. The fabrics were sprayed three times each side using industrial sprayer with the pressure of 140 lb.

Unfinished fabric Chitosan herbal composite finished fabric

Band aids

Band aids or bandages are a strip like piece materials used to cover the wounds so as to provide a moist environment for wound healing process. These materials are also impregnated with microbicides compounds to avoid the entry of infection with surrounding micro flora either from air or the nearby skin. In this study bandages has been developed with chitosan incorporated herbal extract, where the continuous and slow release of the active component and the closed environment would aid in the better treatment for wound healing process.

Bandages:

Bandages are the final medical requirement.

Woven, knitted, or nonwoven and are either elastic or non- elastic materials are used

Common Application

Bandages is to hold dressings in place over wounds.

Leightweight knitted or simple open weave fabrics made from cotton or viscose that are cut into strips then scoured, bleached and sterilized.

Elasticated yarns are incorporated into the fabric structure to impart support and conforming characteristics.

Knitted bandages can be produced in tubular form in varying diameters on either warp or weft knitting machines.

Bandages made of CRAB SHELLS could help wounds heal faster: Mineral taken from crustaceans absorbs liquid and kills bacteria

- **The high-tech alchite dressing is believed to be a world first**
- **Its key ingredient is a mineral called chitosan found in crustacean shells**
- **The mineral is known for healing properties and its ability to kill bacteria**
- **Chitosan has been used in China to treat battle wounds for**

Plasters and bandages could soon be fitted with the shells of crabs to help cuts and scrapes heal faster. The key ingredient in the dressing is a mineral called chitosan found in crustacean shells. It is known for its healing properties as well as its ability to kill bacteria and has been used in China to treat battle wounds for centuries.

Plasters and bandages could soon be fitted with the shells of crabs to help cuts and scrapes heal faster. The key ingredient in the dressing (pictured) is a mineral called chitosan found in crustacean shells. It is known for its healing properties as well as its ability to kill bacteria and has been used in China for centuries The high-tech alchite dressing, believed to be a world first, has been designed by the fibres science and technology team at the University of Bolton, which has patented the product. The team has now developed a commercially viable fast-healing wound bandage and plans to sell it later this year. In ancient China crabs were smashed open and thrust into wounds in battles because chitosan is antimicrobial, meaning it heals and kills bacteria. The team behind the research is led by Professor of Fibre Science and Technology, Mohsen Miraftab, working alongside the Knowledge Centre for Materials Chemistry.

Professor Miraftab said: 'Alchite is a composite fibre, combining alginate, which is drawn from algae, and chitosan, found in crustacean shells.

In ancient China crabs (stock image) were smashed open and thrust into wounds in battles because chitosan is antimicrobial, meaning it heals and kills bacteria. In ancient China crabs (stock image) were smashed open and thrust into wounds in battles because chitosan is antimicrobial, meaning it heals and kills bacteria. The high-tech alchite dressing, believed to be a world first, has been designed by the fibres science and technology team at the University of Bolton

Immersed in water, the composite fibreabsobrbs liquid more than 40 times its own weight (pictured). Alchite can draw liquid away from the wound, and it aids with healing due to its antimicrobial properties ‘Alginate and chitosan both have a history of being used in medicine. Chitosan is naturally antimicrobial and accelerates wound healing activity, so it does heal and kill bacteria.' Professor Miraftab's team is the first to successfully create a combined fibre strong enough to form a wound dressing, through a universally patented technique. Other dressings set to revolutionise the industry include the 'Helix-inspired prosthetic grafts'. Nano prosthetic grafts have been developed which could alleviate the blood vessel-blocking problem associated with fine prosthetic vascular grafts.

The vacuum pump plaster

A new form of plaster which acts like a vacuum pump could help tackle wounds that are hard to heal. The hi-tech dressing sucks out dead tissue, bacteria and debris from the wound that can hinder the healing process and cause infection. At the same time, the vacuum action helps encourage blood flow into the wound, bringing in oxygen and immune cells that speed up the healing process. Numerous studies have shown this technique - known as negative pressure wound therapy - is effective. These grafts would be surgically positioned inside the body to treat a variety of conditions, such as coronary heart disease. Current narrow grafts are prone to calcification and cholesterol build up which eventually closes the fine arteries and could lead to death, but Professor Miraftab's grafts have a unique structure which is based on the helix, a structure at the centre of life - DNA. They are less than 6mm wide and use a technique developed by Professor Miraftab's team. Professor Miraftab said: 'As blood flows through the veins it does not move in a straight line of motion; it spirals. 'By creating a helical structure within the graft we keep the blood moving in its natural path, sweeping the interior of the blood vessel as it moves, hence preventing calcification.' The prosthetic grafts technology will now go for further development and testing with project partner universities and a lead graft manufacturer. Currently Professor Miraftab's team is working on a tendon healing technique which can improve the current surgery options for damaged tendons, replacing with a tendon made from collagen textile-like fibres.

Other dressings set to revolutionise the industry include the 'Helix-inspired prosthetic grafts'. Nano prosthetic grafts have been developed which could alleviate the blood vessel-blocking problem associated with fine prosthetic vascular grafts. This vascular graft shows an internal helix Tendon injuries are common in athletes and are notoriously difficult to heal. The Bolton team has discovered a unique technique to spin collagen fibres, which can be interwoven into the damaged tendon, offering a natural scaffolding support on which cells can grow successfully.

Collagen fibre technology has traditionally lacked the ability to create fibres that can be produced continually and controlled - until now with the university pioneering a highly specialized technique, which produces a continuous, single fine thread, developing a strong structure with controllable flexibility, and on which cells can flourish.

Dimension of the fabric

Length	-	3.4” (8.5 cm)
Width	-	3.9” (10 cm)

Medicated area

Length	-	3.5 cm
Width	-	4.7 cm

Construction details

- The paper pattern was prepared with the given dimension and cut the fabric accordingly.
- The medicated core area was cut in the measurement of in length and breath.
- The medicated area was centrally placed.
- The edges were stick to the sticker sheet.
- Dried in laboratory temperature.

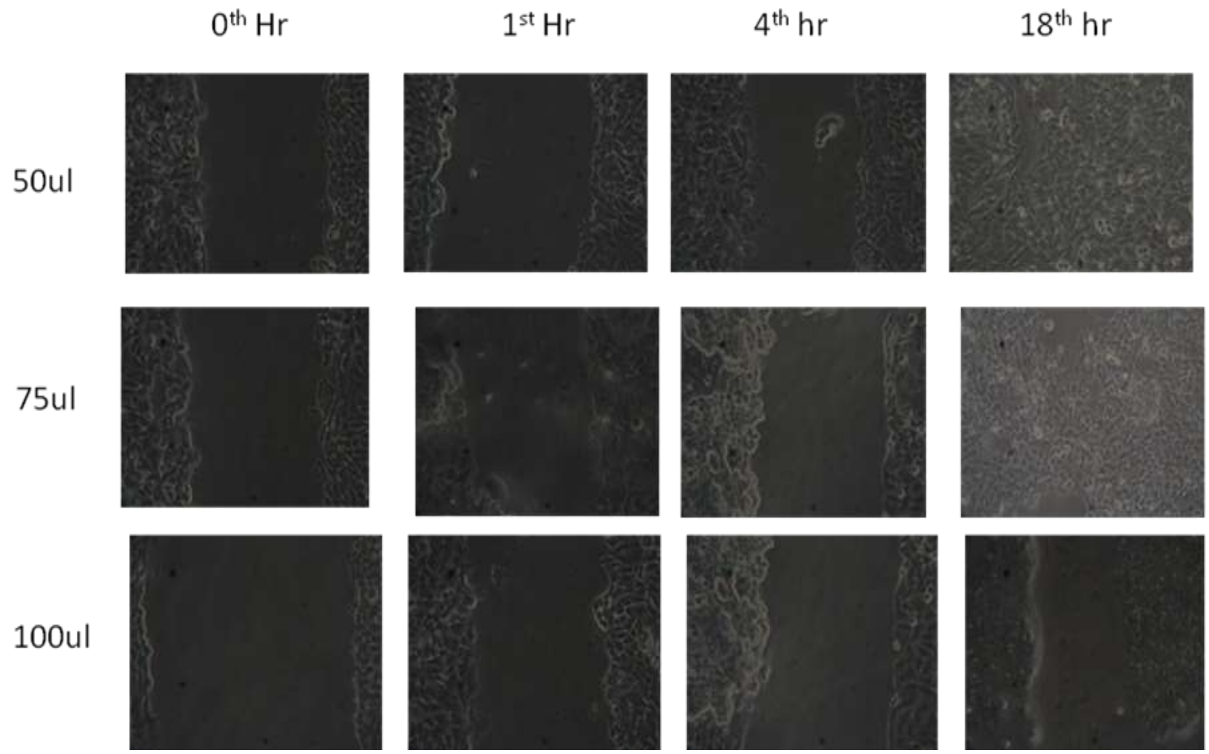
Product development

Step 5: Product development (Wound healing bandage material)

Results and Discussion

Chitosan incorporate with herbal extract (*Aristolochiabracteolata*) showed 80% wound healing activity in 75µL at 18 hrs time period.

chitosan incorporated herbal extract



Plants were inextricably associated with humans from the time immemorial. Earlier the wound treatment was done using the herbal preparations as well as the raw herbs. With the advancement of technology and introduction of allopathic formulation, herbal products have lost their significance. But in the last decade biological, economical and therapeutic benefits of herbal preparations have attracted the world pharmaceutical market in the treatment. Now, once again plants are emerging as time worn invaluable therapeutic agents, with their efficacious wound healing properties. From the results obtained, it is evident that the traditional herbal formulation has significant wound healing activity in the cell line studies and hence, justifying its use in traditional practice. The herbal extracts of *Aristolochiabracteolata* are found to be effective in the functional recovery of the wound healing by dose dependent manner starting from 50 – 100 µl. The result may be attributed to the phytoconstituents present in it which enhance wound healing and provided scientific evidence to the ethnomedicinal futures of *Aristolochiabracteolata*.

There are a number of plants which are used traditionally used the tribal people of India are not been validated or such plants not been evaluated keeping the traditional and conventional claim in mind. Generally pharmacologist should study traditional systems of medicine in scientific way and validate by screening plant/plant extracts for pharmacological activity. This study focused on the wound healing effect by plant extracts and also the development of an acceptable wound healing preparation, which if validated properly and proven scientifically can act as substitute or may even replace the modern wound healing agents. Considering the principle drawbacks, associated with synthetic compounds, plants which are the gift from nature having traditional knowledge, provides excellent raw material for the treatment of various diseases and disorders. As in the allopathic system of medicine, wound healers are available but traditional knowledge in the form of literature provides number of traditional and household preparations for those purposes. Preliminary scientific investigations on plants indicate that natural products could be exploited to discover some novel wound healers.

Thus, all these reported characteristics of ***Aristolochiabracteolata***extracts promote wound healing and also more effective and faster wound contraction. In fact, anti-inflammation, antioxidant and antimicrobial are the main properties of remedies which accelerate wound healing.

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