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A Biomedical Approach for Dvelopment of Wound Healing Bandage Using Silk Fibroin, Manuka Honey And Brahmi

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ABSTRACT: Wound is an inescapable event in life. Wound management is ongoing treatment of a wound by providing appropriate environment for healing. This environment is provided through a wound healing bandage that helps accelerate healing of wound. A variety of wound types has resulted in a wide range of wound dressings with new products frequently introduced. Humans have frequently used plants and natural elements to treat common infectious diseases.Some of these traditional medicines are still part of the habitual treatment as they not only serve merely as a treatment, it is a way of life which has no side effects, gives disease free long life, relaxes mind, makes you tension free while keeping good health status. Research on wound healing drugs/agents from natural resources is a developing area in modern biomedical sciences.Herbal, natural products symbolize safety in contrast to the synthetic products that are regarded as unsafe to both humans and environment. Herbal products have fewer side effects as compared to synthetic, so they are widely used for medicinal purpose. The study is designed to develop a nature based wound healing bandage that helps accelerate wound healing process and has a soothing effect on skin.

Key words: Wound**,** wound management, wound dressing, infectious disease, drugs.

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I. INTRODUCTION:

Wound is an injury involving an external or internal break in body tissue, usually involving the skin [14] which may arise due to physical, chemical or microbial agents. The physiological process of wound healing is a dynamic process and follows a complex pattern of four continuous phases, namely haemostasis, inflammation, proliferation and maturation or remodelling.If not managed properly the wound may become contaminated and infected therefore wound management is a critical process. Proper Management is determined by the wound's size, depth, severity and location over the care period [15]. Wounds get infected by microbes when left open and wet which may be seen as 'pus' formation and a non-healing wound. Therefore, it is important to keep wound area dry and covered with a dressing that keeps microbes away.

Medical textiles is one of the most rapidly expanding sectors in the technical textilesmarket. It is one of the growing areas within technical textiles and the use of textile materials for medical and healthcare products ranges from simple gauge or bandage to scaffolds for tissue culturing and a large variety of permanent body implants. Textile products are omnipresent in the field of human hygiene and medical practice. Their use is based on a number of typical basic textile properties like flexibility, softness, lightness, absorption, filtering.

There is an increasing demand for nature based biomaterials which heal wounds naturally without any side effects and provide a soothing effect to the wound area.

Herbs like Brahmi, Calendula, Cinnamon stick, Echinacea etc. are well known for their antibacterial activities, out of which Brahmi is a special herb that accelerates collagen formation thus enhancing wound healing.

Honey on the other hand is anciently known to treat wounds and burns. All varieties of honey to some extent helps in healing due to their sugar content but some special kinds are used for medical purpose only due to their antibacterial and healing property, these are thus known as medical grade honey or MediHoney. Manuka Honey is one such kind of medical honey.

For a wound to heal in time it is essential for the active components of a wound dressing to reach the wound area to come in action, therefore it is important to have a drug delivery matrix included in the wound dressing which seeps out the active extracts of components present in wound dressing to the wound area. Many natural elements such as chitosan, proteins like silk fibroin are used as a drug delivery matrix in wound dressing. Silk fibroin is a less explored but effective natural component used for the purpose. Silk is composed

of two proteins: fibroin and sericin. Fibroin is the core filament of silk, while sericin is a glue-like protein surrounding the fibres to hold them together in the cocoon case. Silk fibroin (SF) from Bombyx mori silkworm is a fibrous protein which has a long history of use as textiles and surgical sutures. Fibroin, as all fibrous proteins is not soluble in water due to high concentration of hydrophobic amino acids therefore to use silk fibroin as a biomaterial it is essential to convert it into a dispersion which helps in its usage as biomaterial. Gels, membranes or powders can be obtained from Silk fibroin solution. Silk fibroin membranes present a wide range of applications as biomaterials, for instance, wound dressing, drug delivery system or contact lenses.

II. METHODOLOGY:

The current study aims to develop a nature based wound healing bandage that helps accelerate wound healing and has a soothing effect on skin.

Keeping in mind the objectives, a work plan was formulated to carry out the study in following four phases:

Phase 1: Finalising Optimum Concentration of the components

Phase 2: Preparation of silk fibroin biomaterial with potential for drug delivery

Phase 3: Synthesis of non-allergic lanoline

Phase 4: Preparation of bandage

2.1 Finalising the optimum concentrations of the components

2.1.1 Preparation of plant extract

Brahmi powder was collected locally (Delhi,India). Brahmi powder was dissolved in ethanol with different concentrations in a round bottom flask and was kept at room temperature for 3 days in shade. The extract was filtered. The complete procedure was repeated twice with the extract. Different concentrations of Brahmi were taken for which amount of ethanol was kept constant i.e. 20ml. The concentrations made were15.19% ,16.6%,18.36% ,20% and 21.56% of Brahmi.

2.1.2 Preparation of optimum Manuka honey concentration

Manuka honey was sourced from Australia. Hydrogen peroxide, the antibacterial agent is generated by glucose oxidase, an enzyme that bees add to the collected nectar stored in honeycombs. The enzyme is inactive under the low level of free water present in honey, but becomes active if the honey becomes diluted. Therefore, for a 10ml solution honey was dissolved with distilled water in different concentrations. The concentrations were 45%, 50%,55% ,60% ,65% and 70% of water.

All the concentrations of Brahmi and Manuka Honey were tested against bacteria (Staphylococcus aureus, Escherichia coli and Bacillus subtilis)using both Disc Diffusion Method and Agar Well Diffusion Method.

2.2 Preparation of silk fibroin biomaterial with potential for drug delivery

B.moricocoons were sourced from **Central Silk Technological Research Institute(Banglore,India)**. The method used for preparation of silk fibroin based biomaterial was taken from Zhang,H.,et.al[11]. Cocoons from B.mori were degummed by incubating in a mixture of sodium dodecyl sulphate(SDS; 0.25%,w/v) and sodium carbonate(0.25%,w/v) at 98°C for 30mins. The sample were then cooled to room temperature, rinsed three times with distilled water, and dried at 65°C overnight. The ratio of cocoons and solution was 1:100(w/v). the degummed silk fibres were isolated, along with another silk protein, sericin. The isolated fibroin fibres were separately dissolved in concentrated $CaCl_2$solution mixed with ethanol and water (1:2:8 mol at 65°C in a water bath for 4hours. The ratio of the silk fibres and solution was 1:20 (m/v). The aqueous solution of silk fibroin was obtained by dialyzing against flowing water. After that, the resulting dialyzed solution was poured in sterilized falcons covered with parafilm with 5-6holes. The samples were then deep freezed at -80°C for 1hour and then were lyophilized for 48hours in a pre-set lypholizer at -80°C. Dry silk powder was obtained after lypholization. The dry powder was stored at 4°C until use.

2.3 Synthesis of non-allergic lanoline

The method used for the synthesis of non-allergic lanoline was referred from Koresawa et al[11]. The anhydrous lanoline, that is brown in colour, was dissolved in Hexane solution and was then passed through a column containing silica gel. The polar component was absorbed in the silica gel and non polar component solution was collected at the bottom of the column in beaker. This non polar component solution was then heated to evaporate the solvent to give an odourless, white colour refined derivative of lanoline that is non-allergic in nature.

2.4 Preparation of bandage

Final bandage was made by applying primary coating (100ml lanoline, 1% glycerine, 0.5% active charcoal) and secondary coating (0.01gm of silk fibroin derivative per 200µl of Manuka Honey and Brahmi) on a cotton based fabric.

III. RESULTS AND DISCUSSION:

3.1 Selection and evaluation of natural products

having antimicrobial properties

3.1.1 Selection

After reviewing the literature Manuka Honey and Brahmi were selected to use as natural wound healing substances. Various concentrations of these elements with their appropriate reagents were tested against bacteria cultures.

3.1.2 Evaluation

3.1.3.1 Analysis by Agar Disc Diffusion Method

Paper discs impregnated with specific antibiotics or the test substances were placed on the surface of the nutrient agar medium inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard. The plates were incubated and the zone of inhibition around each disc was measured.

TABLE 3.1: Zone of Inhibition by Agar Disc Diffusion Method

NATURAL AGENTS	**CONCENTRATIONS**					
BRAHMI	15.19%	16.6%	18.36%	20%	21.56%	
Bacillus	-	-	-	+	+	
E. coli	-	-	-	+	+	
S. aureus	-	-	-	-	-	
MANUKA HONEY	45% water	50% water	55% water	60% water	65% water	70% water
Bacillus	-	+	+	+	-	-
E. coli	-	-	+	-	+	-
S. aureus	-	-	-	-	-	-

Zone of inhibition observed : +
No zone of inhibition observed : -

Discs of 6mm diameter were prepared using Whatman Filter Paper No. 1 and were autoclaved. These were then dipped overnight in the already prepared concentrations, of Brahmi and Manuka Honey. Petriplates containing 20ml nutrient agar medium were seeded with 18 hours/overnight culture of different bacterial strains and discs were kept on it and a zone of inhibition was observed after overnight and is tabulated as shown in Table 3.1.

The Agar Disc Diffusion test gave a very negligible zone of inhibition, in different concentrations. As the results were not significant and consistent thus it was planned to use the Agar Well Diffusion method for further testing.

3.1.3.2 Analysis by Agar Well Diffusion method

The antimicrobials present in Brahmi and Manuka Honey were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition were uniformly circular as there was a confluent lawn of growth. The diameter of zone of inhibition was measured in centimeters.

The effect of Brahmi and Manuka Honey on several bacterial stains were analyzed by agar well diffusion method.

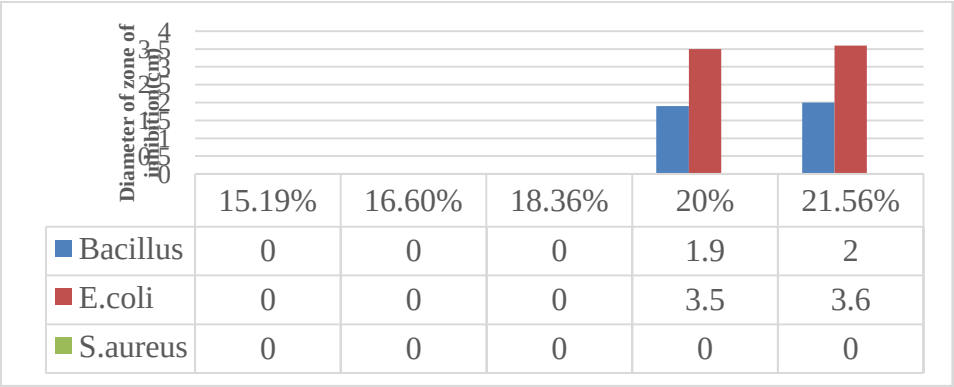
TABLE 3.2: Diameter of zone of inhibition by Agar Well Diffusion Method

NATURAL AGENTS	**CONCENTRATIONS**					
BRAHMI	15.19%	16.6%	18.36%	20%	21.56%	
Bacillus	0	0	0	1.9cm	2cm	
E. coli	0	0	0	3.5cm	3.6cm	
S. aureus	0	0	0	0	0	
MANUKA HONEY	45% water	50% water	55% water	60% water	65% water	70% water
Bacillus	3cm	2.3cm	3.2cm	2.5cm	3cm	2.3cm
E. coli	0	0	3cm	3.2	3.2	0
S. aureus	0	0	0	0	0	0

Petriplates containing 20ml nutrient agar medium were seeded with 18hours/overnight bacterial strains. Wells were cut and 50µl of Brahmi solution and Manuka Honey respectively were added. The plates were incubated at 37°C overnight. The antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).

The zone of inhibition in case of Brahmi is maximum in case of 21.56% concentration, for

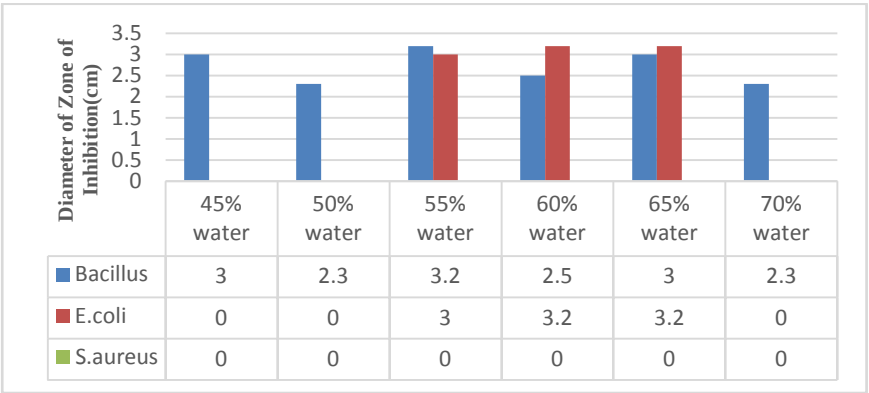
which zone of inhibition is seen for Bacillus and E.coli (2cm and 3.6cm respectively), but the zone of inhibition is similar in 20% concentration, for which zone of inhibition is seen for Bacillus and E.coli (1.9cm and 3.5cm respectively).
Figure 3.1 represents results of zone of inhibition of Brahmi for all concentrations.



	15.19%	16.60%	18.36%	20%	21.56%
Bacillus	0	0	0	1.9	2
E.coli	0	0	0	3.5	3.6
S.aureus	0	0	0	0	0

Figure3.1: Antibacterial activity of Brahmi-Agar well diffusion method

The zone of inhibition in case of Manuka Honey is zero for S.aureus bacterial specie at all concentrations which shows that Manuka Honey does not exhibit antibacterial action against S.aureus. Whereas the zone of inhibition for other bacterial species at all concentrations is in the range of 2.3cm to 3.2cm. Manuka Honey with 55% water shows maximum zone of inhibition for Bacillus (3.2cm). While Manuka Honey with 60% and 65% water shows maximum zone of inhibition for E.coli(3.2cm).
Figure 3.2 represents results of zone of inhibition of Manuka Honey for all concentrations



	45% water	50% water	55% water	60% water	65% water	70% water
Bacillus	3	2.3	3.2	2.5	3	2.3
E.coli	0	0	3	3.2	3.2	0
S.aureus	0	0	0	0	0	0

Figure3.2: Antibacterial activity of Manuka Honey-agar well diffusion method

3.2 Synthesis of silk fibroin biomaterial with potential for drug delivery

The silk fibroins treated with $CaCl_2$-ethanolafter lyphlozation, along with degummed silk fibroins were seen under electronic microscope to observe the morphology (Plate 3.6). The size and shape of the degummed silk fibroins were normal, spherical tubes. In contrast, the regenerated silk fibroins were irregular shaped. This may have resulted from the merger of smaller micelles that occurred in the aqueous solutions of $CaCl_2$-ethanol.

3.3 Formation of non-allergic lanoline

The white non polar compound output was collected in the beaker and polar component of lanoline was soaked by silica gel kept in column.

3.4 Preparation of bandage

The 10cm X 10cm gauze fabric was prepared by primary coating and was further coated with silk fibroin powder, Brahmi and Manuka Honey solution. This fabric was cut into 2cm X 1cm sample pieces. To test the antibacterial property, these prepared sample swatches in both wet and dry states were tested against Escherichia coli (gram negative) and Bacillus subtilis (gram positive). The respective zone of inhibitions were measured, for five consecutive days as shown in the Table3.3.

TABLE 3.3: Diameter of zone of inhibition for the bandage against E.coli and Bacillus

BACTERIA	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
	WET (cm)	DRY (cm)	WET (cm)	DRY (cm)	WET (cm)	DRY (cm)	WET (cm)	DRY (cm)	WET (cm)	DRY (cm)
E.COLI	1.9	2	2	2	2.1	2.2	2.3	2.3	2.3	2.3
BACILLUS	2.7	3	3.1	3.1	3.2	3.1	3.2	3.2	3.2	3.2

On Day 1 the bandage when tested against E.coli , exhibited maximum zone of inhibition in dry state (of diameter 2 cm) as compared to wet state (of diameter 1.9 cm). Similarly, bandage when tested against Bacillus, exhibited maximum zone of inhibition in dry state (of diameter 3 cm) as compared to wet state (of diameter 2.7 cm).

On Day 2 an increase in the diameter of zone of inhibition was observed when the bandage in both dry and wet state was tested against E.coli and Bacillus. The diameters of zone of inhibition were identical in both dry and wet state.

On Day 3 further increase in diameter of zone of inhibition was observed but the diameters were not same for both dry and wet state. When tested against E.coli , maximum zone of inhibition of diameter 2.2 cm was observed in dry state. Whereas when tested with Bacillus, maximum zone of inhibition of diameter 3.2 cm was observed in wet state.

On Day 4 the bandage when tested against E.coli and Bacillus, exhibited equivalent diameters of zone of inhibition for both dry state and wet state (2.3 cm for E.coli and 3.2cm for Bacillus).
On Day 5 no change in the zone of inhibition was observed.

Figure 3.3 and 3.4 show that the bandage was more effective in Bacillus subtilis as compared to Escherichia coli in both dry and wet state. Further no significant difference was observed in the effectiveness of bandage in dry and wet state, both became equally effective on Day 4 in case of both groups of bacteria.

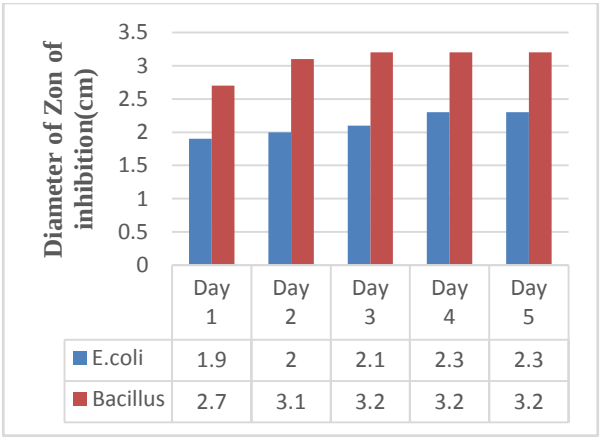


Figure3.3: Antibacterial activity of wet bandage against E.coli and Bacillus by Agar Diffusion Method

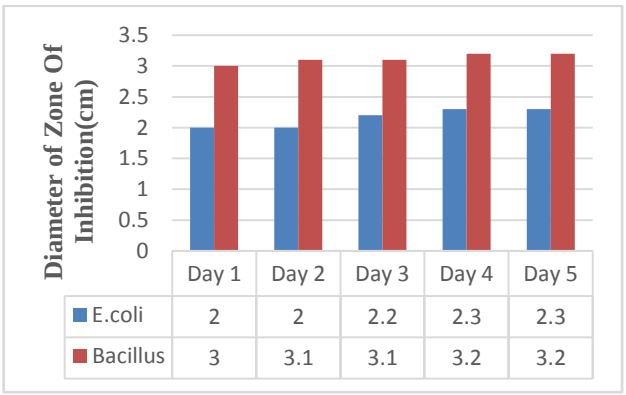


Figure3.4: Antibacterial activity of dry bandage against E.coli and Bacillus by Agar Diffusion Method

Above results indicate that this bandage is effective in controlling infection due to both gram negative and gram positive bacteria till the end of fourth day.

IV. CONCLUSION:

Wound healing is a biological process which relates to general phenomenon of tissue regeneration. Wound healing process, is a series of interdependent and overlapping stages, in which a variety of cellular matrix components act together to re-establish the integrity of damaged tissue and replacement of lost tissue. Plants and other natural derivatives have been a source of medicinal compounds and play an important role in maintain human health since ancient times.These nature based components help healing wound without having any side effects. They have a soothing effect on skin.

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