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Research Article

Development of Film Using Biopolymer and Herbal Extract for Biomedical Application

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Abstract

The current study aims at the development of film using herbal extract (*Vitex negundo*) and biopolymer (sodium alginate) for biomedical application. The plant with potent inflammatory property (*Vitex negundo*) was selected for the study. The leaf extract of *Vitex negundo* was immobilized on the polymer to enhance the antimicrobial activity. The films were prepared by solvent-casting method followed by cross-linking to improve their properties in dry and wet condition. The prepared film using herb and biopolymer showed good antimicrobial properties in dry condition with 100%, 75%, and 50% bacterial reduction compared to the control sample, while in wet condition there is no difference in bacterial reduction.

Keywords: Antibacterial Activity; Sodium Alginate; Solvent Casting; *Vitex negundo*; Wound Healing Film

Introduction

Medicinal plants have been identified and used throughout human history. *Vitex negundo* is very popular as a medicinal plant as mentioned in the ancient text of ethnics. The leaves produce a cooling effect that eases pain and swelling [1]. The leaves were found to possess anti-inflammatory property and hence selected for biomedical application. Sodium alginate is one of the biopolymer and also it has anti-microbial property. Alginate is a natural polymer widely used for the treatment of several types of wounds, due to its biocompatibility, biodegradability and ability to form film.

Films are very flexible and are good for wounds on difficult anatomical sites for example, over joints[2].Alginate is a polysaccharide belonging to the family of linear (un branched), non-repeating copolymers, consisting of variable amounts of β-d-mannuronic acid and its C5-epimer α-l-guluronic acid linked via β-1,4-glycosidic bonds. Like DNA, alginate is a negatively charged polymer, imparting material properties ranging from viscous solutions to gel-like structures in the presence of divalent cations [3] . Alginates possess good antimicrobial properties, ion-exchange and gel-forming abilities which makes it suitable for biomedical applications.

Materials and Methods

Selection and Extraction of Herb

Vitex negundo has been identified as a plant with medicinal properties. The leaf extract is rich in antioxidants, phenolics and flavonoids; presence of many phytochemicals and vitamin E contributes properties like antimicrobial, antioxidant, anticancer, hypercholesterolemic, antiulcarogenic, etc[4]. The leaves were collected and washed thoroughly in running water. Then the leaves were dried under shade and ground to fine powder.

- *Vitex negundo* leaf powder - 50 grams
- Methanol - 500 ml
- Temperature - 50ºC
- Time - 48 hours

The dry powder was extracted using Soxhlet apparatus with methanol as solvent. 50 grams of *Vitex negundo* leaf powder is

packed in 100% cotton cloth. Then 500 ml of methanol is poured in apparatus and refluxed for 48 hours. Finally, the extract is taken from the flask and filtered using Whatman No.1filter paper and the extract has been redistilled to enhance the concentration to 25%.

Alginate - Biopolymer

Alginates are produced from the naturally occurring calcium and sodium salts of alginic acid found in a family of brown seaweed (Phaeophyceae). They generally fall into one of two kinds: those containing 100% calcium alginate or those that contain a combination of calcium with sodium alginate, usually in a ratio of 80:20. Alginates are rich in either mannuronic acid or guluronic acid, the relative amount of each influencing the amount of exudate absorbed and the shape the dressing will retain. Alginates partly dissolve on contact with wound fluid to form a hydrophilic gel as a result of the exchange of sodium ions in wound fluid for calcium ions in the dressing. Those high in Mannuronic Acid (such as Kaltostat) can be washed off the wound easily with saline, but those high in Guluronic Acid (such as Sorbsan) tend to retain their basic structure and should be removed from the wound bed in one piece[2].

Sodium alginate has been identified as a biopolymer with medicinal properties and it has a tendency to form a film. It has natural anti-microbial property and also wound healing property.

Preparation of Film

The films were prepared through the solvent-casting method and subsequently submitted to an additional cross-linking step to improve their properties [5].

- Sodium alginate- 0.375 grams
- Distilled water- 25ml
- Glycerol- 0.15 m/ml
- Herb extract- 1 ml (25% concentration)
- Calcium chloride- 5 grams for 100ml of distilled water

For the preparation of film, 0.375 grams of sodium alginate is taken and dissolved in 25ml of distilled water. Then glycerol (0.15m/ml) is added with dissolved solution to enhance the flexibility of the film. Then, 1 ml of alginate is removed and 1 ml of extracted herb is added to enhance the property.

Apetridish is cleaned with cotton dipped in surgical spirit to avoid contamination, if any. Then, the prepared solution is evenly poured into the plate and left for drying at room temperature. Finally, after drying the film is taken from the plate and dipped into Calcium Chloride ($CaCl_2$) for 5 minutes for ionic-cross linking on the film and left to dry. (Figure 1)

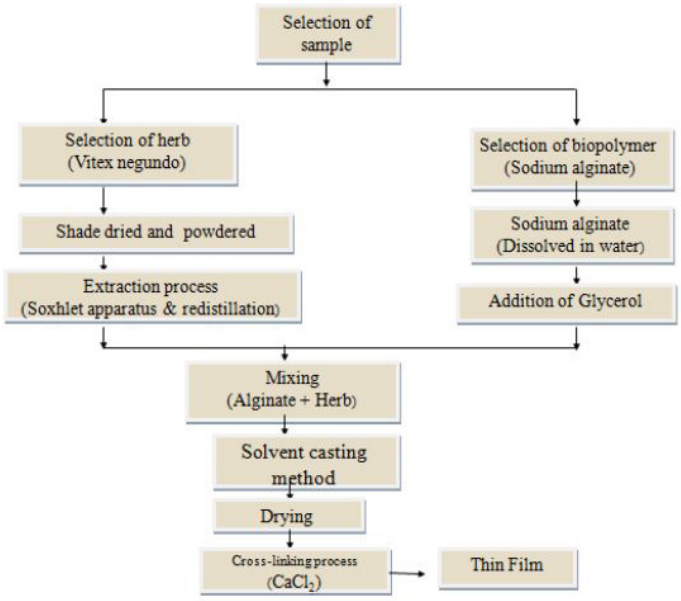

Figure 1: Experimental Procedure.

Percentage of Inhibition

$$\text{Percentage of inhibition} = \text{ZOI in treated} - \frac{\text{ZOI in control}}{\text{ZOI in treated}} \times 100$$

Results and Discussion

Antimicrobial Activity of Treated Sample

Antimicrobial activity of the prepared film sample is to be determined. Muller Hinton agar (MH) medium was prepared for the test. Medium size petridishes, cotton waskept in autoclave for 3 hours. The medium is poured into the plate and left to dry for 30 minutes. Then, the organisms such as *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* were taken from agitator and swapped over the medium using cotton. Then, the sample is taken in wet and dry conditions. Both samples were cut in equal diameters into small pieces (2 X 2) and placed over swapped places. Then the plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the zone of inhibition of each sample was measured.

Zone of Inhibition [ZOI]

ZOI is an area of growth inhibition around a point source, within a lawn of cultured organisms on a solid medium, due to the action of a growth-inhibitory substance, such as an antimicrobial agent, present at the source [6]. From (Table 1) and (Figure 2), it could be inferred that the wet sample did not show any difference in antibacterial activity of the control and the herbal treated film.

(Table 2) and (Figure 3) shows the antibacterial activity of the dry film, where improvement is noted in the herbal treated film compared to the control film. Hence, the film is suitable for dry wounds than wet wounds. (Figure 4)

Sample	Zone of Inhibition- ZOI (in cm)		
	Escherichia coli	***Staphylococcus aureus***	***Streptococcus pyogenes***
Treated with herb (Wet)	2	2	2
Control(Wet)	2	2	2
Treatedwith herb(Dry)	2	2	2
Control(Dry)	1	No Inhibition	0.5

Table 1: Zone of Inhibition [ZOI].

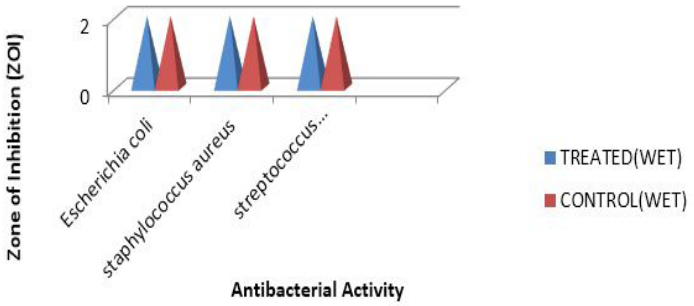

Figure 2: Bar chart for ZOI (wet sample).

Sample	Percentage of Inhibition %		
	Escherichia coli	***Staphylococcus aureus***	***Streptococcus pyogenes***
Treated (dry)	50	100	75
Treated (wet)	Nil	Nil	Nil

Table 2: Percentage of Inhibition.

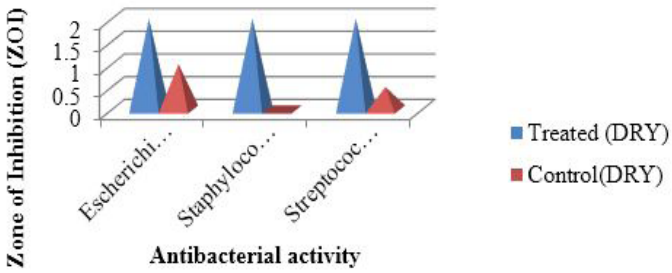

Figure 3: Bar chart for ZOI (dry sample).

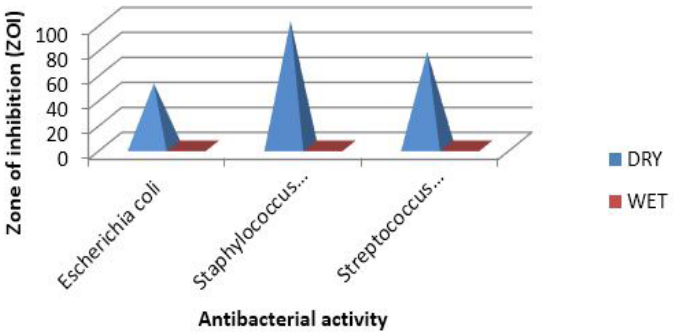

Figure 4: Bar Chart for Percentage of Inhibition.

Conclusion

Alginate gels are used in wound healing applications and still could be improved by addition of herbal extracts with good healing properties. Wound healing film developed with alginate and *Vitex negundo* leaf extract possess better antibacterial property in dry condition compared to the wet condition. The film is suitable for both dry and wet wounds. The film needs a suitable secondary dressing to keep intact with the wound. Alginate wound dressings have excellent scope in medical industry and hence research prospects is also abundant.

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