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Research Article

Antimicrobial Activity of Aloe Vera Formulation

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ABSTRACT

With the increasing incidence of diseases and development of antibiotic resistance, the alternative, safe, effective, and economical products are the need of time. Proven in multiple clinical studies, Aloe vera has been used in antimicrobial activity and used in mouth ulcers, periodontal diseases etc. In this present study Mucoadhesive film was prepared to study antimicrobial activity, to improve therapeutic efficacy by its adhesive action. The present study discusses the estimation of the antimicrobial activity of Aloe vera loaded mucoadhesive films. The formulation was studied for percent inhibition of *microorganism of inflammed tissue, Calculas,* and *cultures of S.oralis, C.albicans, S.mutans.* The formulation showed Percent inhibition of *Candida albicans* more when compared with standard Chlorhexidine. The film prepared is economic, convenient and shows the antimicrobial activity.

Key-words: antimicrobial activity, Aloe vera, mucoadhesive film.

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INTRODUCTION

Aloe gel is the clear, jelly-like substance found in the inner part of the aloe plant leaf. Aloe latex comes from just under the plant's skin and is yellow in color. Aloe vera contains 99.3 percent water and 0.7 percent nonaqueous constituents, such as glycoproteins and polysaccharides etc. Glycoproteins speed the healing process by stopping pain and inflammation[1], while polysaccharides stimulate skin growth and repair[5,6]. These substances may also stimulate the immune system.

It is said to be a natural cleaner, powerful in penetrating tissues, relieving pain associated with joints and muscles, bactericidal, a strong antibiotic, virucidal when in direct contact with long periods, fungicidal, anti-inflammatory[4], instrumental in increasing circulation to the area, breaking and digesting dead tissue and moisturizing tissues. The strongest antibacterial effect is found in extracts of the aloe leaves and in the gel made from the inner leaves. It contains glycoproteins and polysaccharides etc. Aloe vera is one such product exhibiting antimicrobial activity [7,8,9]. Recent advances in the field of dentistry have promoted the use of aloe vera for treatment of various oral diseases and periodontal conditions[10,11,12]

Objectives:

To evaluate the antibacterial activity of prepared herbal formulation against *microorganism of inflammed tissue,Calculas,and cultures of S.Oralis,C.Albicans,S.Mutans.*

EXPERIMENTAL

Materials:

1.The *aloe vera* leaves (*Aloe Barbadensis,* Family: Liliaceae) were procured from Nisargamitra Aloe Vera Farm-Daheri, Umbergaon, Gujarat

2. Chlorhexidine gluconate was gift sample from Unilab Pvt Ltd.

MATERIALS AND METHODS

Extraction Procedure: Juice Collected after Roller Pressing

Plant material was collected and washed with distilled water. The leaf was cut into cubical pieces (chunks). The upper and lower skin of each leaf was then carefully removed with the help of knife and the mucilaginous mass made up of parenchymatous tissue (filets) was cut into small pieces. Care was taken to avoid contamination of yellow sap with the filet.

Filets were inspected visually for any contamination or adhering yellow sap or any outer green skin and discarded if necessary. The total mass of the uncontaminated fillet was approximately about 40% of the leaf

mass.

The uncontaminated filets were homogenized twice in a blender at a high speed for 5 minutes. The foam was developed which was settled after 10 minutes. The extracted gel was subjected to straining through muslin cloth to remove traces of cellular matter. The clear gel was then subjected to further processing.

Preparation of mucoadhesive film:

1. Weighed quantity of Aloe Vera juice was taken and kept at room temperature for 10-15min (since juice was taken from refrigerator).
2. HPMC was dissolved in 10ml of distilled water with gentle stirring.
3. Acrylate copolymer solution was prepared in ethanol into another beaker.
4. To HPMC dispersion, Eudragit RLPO solution was added and then stabilized Aloe Vera juice with respective conc. was added along with PEG 400 was added and stirred well.
5. Homogenised solution was solvent casted on petri plate at 60^0C[1].

HPMC	Aloe Vera Juice	Acrylate copolymer	PEG 400
1gm	10ml	1gm	0.3ml

Table 16: Formulation of Aloe Vera loaded films

Result: Film was formed with smooth surface.

Study of antimicrobial activity:

Sample preparation: A placebo Mucoadhesive film as well as Aloe Vera loaded films were dissolved into 10ml of distilled water and kept for stirring on magnetic stirrer for 3hrs. After complete dissolution of the films the solution was filter through Whattman filter paper and resulting filtrate was used as a sample. Simultaneously agar plates were prepared with respect to microbial strains and after solidification; agar plate was bored with the help of cork borer and samples poured into bore. After 24 and 48 hours' zone of inhibition was measured in mm.

Preparation of medium:

1. **For *Streptococcus mutans*, inflamed tissue, calculus Mutans-Sanguis Agar M977 was used.**

Media preparation: Suspend 98.1 grams in 1000 ml distilled water. Heat to boiling and to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into Petri plates.

2. **For *Candida albicans,* Candida Agar was used**

Media preparation

Suspend 41.1 grams in 1000 ml distilled water. Heat to boiling with frequent agitation to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates

3. **For S oralis, Ready plates of Tryptic soya agar with 5% sheep blood was procured from Hi Media and used**

1. 3 Microorganism and media:

- The test organism used in study was *Streptococcus oralis* (MTCC No.2696), *Streptococcus mutans* (MTCC No.497), and *Candida albicans* (MTCC No.227) procurred from MTCC Chandigarh and cultures from inflamed tissue, and Calculus from Nair Hospital, Mumbai Central.
- All media used were sourced from Hi-media Laboratories.

1. 4 Antimicrobial activity[2, 3,13]: Well Diffusion Method" was adopted for evaluation of activity: *Porphyromonas Gingivilis (inflamed tissue), Methanobrevibacter oralis, and Streptococcus mutans from (Calculus)* were incubated in Mutans Sanguis agar for 48 hours under anaerobic conditions and adjusted to yield approximately 1.0×108 CFU/ml. For *Streptococcus oralis,*

a prepared inoculum was added to the molten agar, mixed and poured over the surface of the agar base (Room temperature) and left to solidify.

Tryptic Soya agar (5 % sheep blood) was used for *Candida albicans,* candida agar was used under aerobic conditions and incubated for 24 hours at 37°C and adjusted to yield approximately 1.0×108 CFU/ml. The procedure was the same as performed above, except the plates were incubated at 37°C for 24 hours under aerobic conditions.

All the Well Diffusion tests were performed in duplicate and antibacterial activity was expressed as the mean of inhibition diameters (mm).

A well approximately 9 mm in diameter was bored on the surface of agar medium using a sterile cork borer.

Then the sample was introduced into the well. Chlorhexidine (200mg/ml) was used as the standard.

The plates were then incubated at 37ºC for 48 hours under anaerobic conditions in an anaerobic jar (Hi-Media) with gas pack and indicator tablet and the jar was kept in an incubator for 48 h at 35.5 ± 1ºC.

Anaerobic gas pack - a disposable oxygen absorbing and carbon dioxide generating agent for use in anaerobic jar was used to maintain and check the anerobiosis. Anaerobic tablet having pink color was used, on introduction into the jar color remains pink indicating anaerobic conditions (original pink color if change to purplish-blue indicating aerobic condition because of the absorption of oxygen).

1. 5 Measurement of zone of inhibition The zone of inhibition for each sample was observed, measured and expressed in mm. From this the activity index (A.I.) and Percent Inhibition (P.I.) were calculated.

A.I = Mean zone of inhibition of each solvent extract/Zone of inhibition obtained for standard

P.I. = Activity index x 100

RESULTS and DISCUSSION

Results of antimicrobial activity of Aloe Vera loaded films (In term of zone of inhibition in mm)

Organisms	Standard (mm)	Blank	Aloe Vera Loaded films (mm)
S. mutans	9.6	NIL	8.9
Candida albicans	8.5	NIL	8.4
S. oralis	12.3	NIL	8.8
Inflame tissue	8.6	NIL	8.1
Calculus	11.3	NIL	8.2

Table 1: Antimicrobial activity of Aloe Vera loaded films

Results of antimicrobial activity of Aloe Vera loaded films (In term of Percentage of Inhibition)

Organisms	Standard	Blank	Aloe Vera Loaded films
S. mutans	100	NIL	92.70
Candida albicans	100	NIL	98.82
S. oralis	100	NIL	71.54
Inflame tissue	100	NIL	94.18
Calculus	100	NIL	72.56

Table 2: Antimicrobial activity of Aloe Vera loaded film

- In the present study formulation was examined for antimicrobial activity by Zone of inhibition. Aloe vera loaded film showed zone of inhibition for all microorganism species studied.
- Results showed that Percent Inhibition of the formulation for Candida albicans was found to be better than standard i.e. Chlorhexidine.

CONCLUSION:

Aloe vera juice is a potential candidate for antimicrobial activity, especially in mouth infections. Hence Mucoadhesive films of Aloe Vera can be used as a targeted drug delivery system to treat the periodontal conditions.

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