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FORMULATION AND EVALUATION OF FILM FORMING GEL OF PUMPKIN LEAVES EXTRACT FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Bacterial infection of the skin is one of the most common dermatological problems. Delivery of drugs to the skin is an effective and targeted therapy for local dermatological disorders. In the present study film-forming gels of pumpkin leaves extract were formulated for treating bacterial infections of the skin. The extraction of pumpkin leaves was done by maceration method and from the extract two different film forming gel formulations were prepared by using Carbopol 934 as gelling agent, PVP & PVA as film formers and other additives. The prepared film-forming gel formulations were evaluated for physical appearance, pH, viscosity, spreadability, extrudability, drying time to form film, thickness and antibacterial activities. The formulations exhibited satisfactory results for the evaluated parameters. Both formulations found to show satisfactory antibacterial activity i.e 20±0.81mm(F1) and 19±0.47mm (F2). By considering these factors it was concluded that the film-forming gels containing pumpkin leaves extract can be used for the treatment of bacterial infections effectively.

KEYWORDS: Film-forming gel, maceration method, pumpkin leaves extract, antibacterial activity.

INTRODUCTION

Topical skin infections commonly occur and often present therapeutic challenges to practitioners, despite the numerous existing antimicrobial agents available today. The necessity for developing new antimicrobial means has increased significantly due to growing concerns regarding multidrug resistant bacterial, viral, and fungal strains. The common topical skin infections are due to the growth of microorganisms including *Candida*, *Staphylococcus*, *Streptococcus* species etc.[1]

The goal of drug administration through skin is for topical treatment of skin disease or for transdermal absorption of drug.[2] The localized treatment of disease requires that the pharmaceutical active ingredient be maintained at the site of treatment for an effective period of time. The dosage forms such as ointments, gels and creams are associated with several limitations, most commonly getting washed away quickly by contact with water. This necessitates longer treatment duration. Hence, to overcome the problems associated with conventional topical dosage forms, film forming systems has been developed. It is a novel approach which can be used as an alternative to conventional topical and transdermal formulation.[3,4]

Film forming gels which on application forms a thin, transparent film on skin surface. Film forming gels when compared to conventional semisolid topical products, are aesthetically more attractive to patients. They are non-sticky, adhere to the affected part for a longer period without getting rubbed off and can be designed to provide sustained drug release so that frequent reapplication is not required. After application to the skin, it forms film due to the loss of volatile components of the vehicles which results in formation of residual film on the skin surface.[3,4]

Pumpkin leaves are from thread like green plant that usually has a big fruit with a hard cover. The aqueous extract of pumpkin leaves had been reported to reduce blood glucose level. Various reports also proved that pumpkin leaves extract can inhibit bacterial species like *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* etc and fungal species like *Aspergillus niger*, *Candida albicans* etc.[5,6]

Hence, the present study focused on the formulation of a safe herbal formulation for treating the topical bacterial infections by developing film forming gels containing extract of pumpkin leaves.

MATERIALS USED

Pumpkin leaves extract was obtained from local farm, Mangalore. Carbopol 934, PVA, PVP, Propyl paraben, PEG 400, Propylene glycol were obtained from Hi-Media laboratory Pvt Ltd, Mumbai. All ingredients used in the preparation were of analytical grade.

METHODS

Extraction of pumpkin leaves[7]

Fresh pumpkin leaves were collected and air dried at room temperature over a period of one week. The leaves were reduced to powder using an electric blender. The dried leaves were extracted using methanol for 72 hours with constant agitation after which it was filtered using filter paper. The filtrate was evaporated to dryness using an electrical water bath at 100^0C. The extract was then stored inside the refrigerator at 4^0C for further use.

Preparation of film forming gel containing extract of pumpkin leaves[6,8]

Table 1: Composition of film forming gel.

Ingredients	Formulation Code	
	F1	F2
Pumpkin leaves extract(g)	0.5g	0.5g
Carbopol 934(g)	0.4g	0.4g
Polyvinyl alcohol(g)	4.28g	4.28g
Polyvinylpyrrolidone (g)	0.71g	1.71g
Ethanol(ml)	5ml	5ml
Poly ethylene glycol 400(ml)	2.5ml	2.5ml
Propyl paraben(g)	0.05ml	0.05ml
Tri Ethanol Amine(ml)	q.s	q.s
Distilled water (q.s for 50ml)	q.s	q.s

To prepare film forming gel, appropriate amount of Carbopol 934 was soaked in water for a period of 2 hours. Weighed amount of PVA and PVP in different concentrations were mixed and dissolved in distilled water by heating on water bath with continuous stirring until a homogenous mixture was formed. This was then transferred to Carbopol 934 gel.

Weighed quantity of pumpkin leaves extract was taken to which ethanol, PEG 400 and propyl paraben were added under continuous stirring by a magnetic stirrer, to form a homogenous mixture. The above gel was then neutralized with triethanolamine (TEA) and the volume was made up to 50 ml by distilled water.

EVALUATION OF FILM FORMING GELS

Prepared film forming gel formulations were subjected to various evaluation parameter as follows;

Physical examination[9]

Prepared gels were inspected visually for their appearance, homogeneity and consistency.

pH[10,11]

pH measurements were carried out on the gels using a digital type pH meter, by inserting the glass electrode completely into the gel system and waiting for stabilization of the pH.

Spreadability[9,12]

To determine the spreadability, approximately 1gm of the gel was placed between two glass slides of same dimensions (20cm*20cm). A weight of 1000gm was placed on the upper glass slide and allowed the gel to spread. After 1min the weight was removed, and diameter of spread area was noted.

Extrudability[9,12]

The gel formulations were filled in standard collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and weight of 1kg was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The % of gel extruded was calculated, recorded.

$$\%\text{Extrudability} = \frac{\text{Initial weight of the tube(g)} - \text{extruded weight(g)}}{\text{Initial weight of the tube(g)}} \times 100$$

Viscosity[9,12]

Viscosity of the gel was determined by using Brookfield viscometer (LDVD E). Required quantity of the gels were taken in a beaker and spindle number 64 was used at rpm of 1 and 5 for F1 and F2 respectively and the viscosity was noted.

Drying time[13]

One gram of the gel was placed and spread uniformly on a petri dish. It was subjected for drying by keeping it in a hot air oven at 37˚C and time needed for the gel to convert into film was measured.

Film thickness[14]

The thickness of the film was evaluated using screw gauge. Anvil of the thickness gauge was turned and the film was inserted after making sure that the pointer was set to zero. The film was held on the anvil and the reading was noted.

In-vitro antimicrobial susceptibility study[9]

The agar-well diffusion method was used to determine the antimicrobial activity of the formulated gels. Nutrient agar media was mixed with 24hrs old test culture and poured into sterile culture plates and allowed to solidify. With the aid of sterile cork borer, well was punched on the plate. Formulation containing the pumpkin leaves extract was aseptically transferred into the wells and plate was incubated at 37˚C for 24 hrs. Then zone of inhibition was measured after 24 hrs and recorded.

RESULTS AND DISCUSSIONS

Gel formulations were inspected visually for the physical appearance. It was found that the formulations were having green color and excellent consistency and good homogeneity.

pH

pH of the film-forming gels was determined by using digital pH meter. From the results (Given in Table No. 2) it was found that pH value was 7.35 and 7.36, which is compatible to the skin pH and is considered satisfactory for application with minimal risk of tissue irritation.

Viscosity and spreadability
The obtained results for viscosity and spreadability determination are given in the table number 2. The value of the spreadability indicated that the film-forming gel were easily spreadable.

Extrudability
Extrusion of the film-forming gel from the tube is important during application and for the patient compliance. The results obtained for extrudability studies is shown in table number 2.

Table 2: Results of pH, Viscosity, Spreadability and Extrudability.

Formulation code	pH	Viscosity (cps)	Spreadability (cm)	Extrudability (%)
F1	7.36± 0.02	13,099 ± 0.94	6.7± 0.081	89.46± 0.088
F2	7.37± 0.02	13,300 ± 0.47	7.1± 0.081	87.26± 0.252

Drying time
Drying time is very important after application to know at what time the drug will get dried on the skin. The obtained results of drying time are shown in table number.3. It is observed that the concentration of polymer affects the drying time. As the concentration of film forming polymer increases, time required for the film to form decreases.

Thickness
Thickness of the film was measured by using screw gauge. The values obtained during evaluation are shown in table number.3. Both formulations found to show the uniform thickness as there is no much difference in the concentration of polymeric solution.

Antibacterial activity
Antibacterial activity was checked by agar well diffusion method and zone of inhibition was measured using antibiotic zone reader. The results are shown in table number 3 and figure number 1. From the results it was found that both formulations have shown satisfactory inhibition of bacterial growth.

Table 3: Results of Drying time, Thickness and Antibacterial activity.

Formulation code	Drying time (min)	Thickness (mm)	Zone of inhibition (mm)
F1	15 ± 0.2	0.01± 0.008	20 ± 0.81
F2	12.26 ±0.2	0.02±0.008	19 ± 0.47

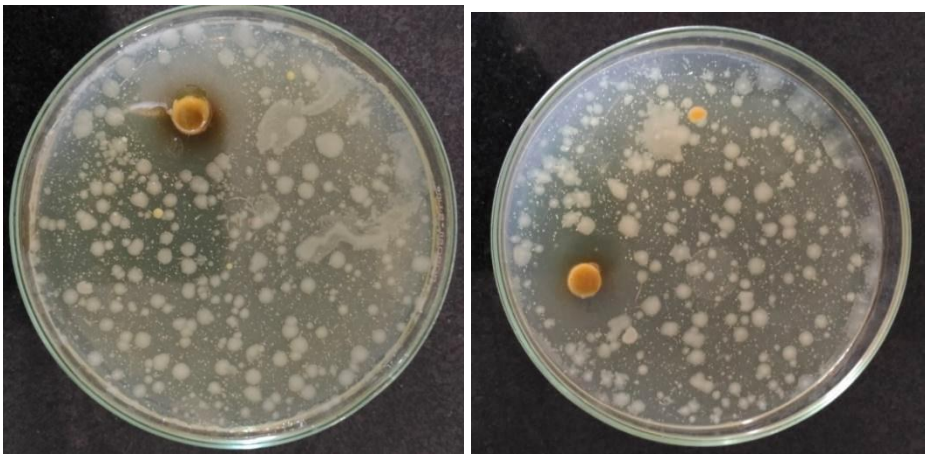

Figure 1: Antibacterial activity of F1 and F2 formulations.

CONCLUSION
Film forming gels of pumpkin leaves extract were formulated successfully by using gelling agent like Carbopol 934 and polymers like PVA, PVP in different concentrations. All the prepared formulations were subjected to various evaluation parameters and obtained results were most satisfactory. Antibacterial activities of the formulations were also found to be appreciable.

Hence, the preparation and evaluation of film forming gels of pumpkin leaves extract was a successful approach for overcoming the demerits associated with conventional semisolid topical dosage forms. These formulations are also required for the improved patient compliances.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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