EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF LOTION CONTAINING ALOE VERA, NEEM EXTRACT AND GARLIC OIL

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ABSTRACT: The aim of this study to develop a topical lotion formulation of herbal extract containing garlic oil, aloe vera and Neem extract with different concentration of polymer. Topical lotion formulation was designed using Stearic acid and triethanolamine with different ratio were chosen as independent variables while pH and viscosity as dependent factors. The lotion was prepared by adding non-polar phase to polar phase with rapid stirring to avoid separation of water and oil phase. Non-polar phase was first melted together and slowly added to the preheated mixture of polar phase. Stearyl alcohol in combination with other ingredients in the formulation such as triethanolamine and stearic acid forms an emulsion.
Three different types of formulation oil in water (o/w) ointment namely F1,F2, F3& F4 where formulated by using different Herbal extract, concentration of polymer and propylene glycol solvent. The prepared lotion was evaluated for different parameter such as organoleptic properties, pH, practical yield, irritancy, washability and phase separation etc. The evaluation for Antibacterial and Antifungal activity was done using cup plate method. The lotion showed promising Antibacterial and Antifungal activity against other strains used for the study. The lotion was stable at room temperature. The formulation showed no redness, edema, inflammation and irritation that indicated that it is safe to use Then the F3 and F4 formulation showed better consistency and spreadability when compared to F1 and F2 formulation..

KEYWORDS: Aloe vera, *Azadirachta indica,* Garlic oil, Antifungal, Anti bacterial and Lotion

INTRODUCTION

Fungal infection of the skin is nowadays one of the common dermatological problems. The physicians have a wide choice for treatment from solid dosage to semisolid dosage form and to liquid dosage formulation. Among the topical formulation, clear lotions have widely accepted in both cosmetics and pharmaceuticals.

Since the vedic era humans uses medicinal plants material for to cure any disease or to give a satisfactory treatment against that disease. It is like to be says that there is none of a plant on the earth which is without any medicinal property. from this it is shows that the importance plant or a part of a plant a medicine i.e Herbal medicine. The purpose of the current study is also based on the medicinal property of a plant i.e. Garlic (*Allium sativum*), *Aloe vera and Azadirachta indica*

Garlic oil is shows a wide range antimicrobial activity[3]. Alliin is present in the garlic oil, when Garlic cloves are crushed then enzyme Allinase is converted alliin into the allicine and allicine is again forms many sulphide compounds. Garlic oil consist of sulfur containing compounds such as allicin, alliin, ajoene, diallyl disulfide, dithiin and Sallyl cysteine. Diallyl disulfide is an important component in the garlic and being a powerful antibiotic and antifungal compound,

Aloe vera (family- Liliaceae) is a stem less plant. A. vera reported to contain mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals. Traditionally, A. vera has been used in ointments and creams to assist the healing of wounds, burns, eczema, and psoriasis1. It has also been used in anti aging and anti wrinkle creams and moisturizers. It has been also reported to have antifungal properties. *Azadirachta indica* (Family-Meliaceae) known as Neem is well known for its medicinal properties. Neem has been highly successfully against harmful fungi, parasites, and viruses. It has been most helpful in treating a variety of skin problems and diseases including gzpsoriasis, eczema and other persistent conditions. Its leaves possess broad spectrum of activity against Gram +ve and Gram –ve bacteria including M. tuberculosis, Vibrio cholera.

Numbers of disease caused by fungus are Aspergillus, blast mycosis, candidacies, and histoplasmosis. In modern years the incidence of these infections has increased steadily, mainly because of the rising number of immunocompromised patients and the growing status of health clubs and public swimming pools, which simplify the spread of infection.

MATERIALS AND METHODS

Collection of Materials : Propylene glycol, bees wax, stearyl alcohol, cetyl alcohol was purchased from Research-lab fine chem Industries Mumbai. Triethanolamine, propyl paraben, methyl paraben, liquid paraffin was obtained from Pure chem laboratories Pune. stearic acid was purchased from Ozone International Mumbai.

Preparation of plant extract: The *Aloe vera* leaf was cut at the base of the plant, lower leaf was sliced opened peeling the outer portion of skin and pericarp and juice was collected. The neem leaves were also dried and powder was percolated with 250 ml of 80%ethanol. The volume of plain *Aloe vera* juice was kept constant in each formulation. Weighed quantity of each of neem leaves extract and garlic oil was mixed with *Aloe vera* juice before adding the polymer[4].

Preparation of cream formulation

Preparation of oil phase : White Bees Wax, stearic acid, stearyl alcohol, cetyl alcohol were melted in a stainless steel vessel. To this mixture Liquid paraffin were added and allowed to melt. The temperature of oil phase maintained between 65 – 70°C[3] .
Preparation of Aqueous phase : Water was heated to 65 – 70°C. In this weighed propylene glycol, triethanolamine, methyl paraben and propyl paraben were added the temperature of the phase was maintained at 65 – 70°C. Both oil and aqueous phase ingredients listed in table 1.
Development of Lotion formulation : Oil portion was then slowly incorporated into the aqueous phase at 65-70°C and mixed for 10 to 15 Minutes. When the water and oil phase were at the same temperature, the aqueous phase was slowly added to the oil phase with moderate agitation and was kept stirred until the temperature dropped to 40°C. and mixture of plant extract was added to it. The emulsion was cooled to room temperature to form a semisolid cream base. pH of cream kept between 4.5 – 6. Formula for given lotion given in table no 1&2.

EVALUATION PARAMETER

Organoleptic characteristics : Drug-loaded formulations were tested for physical appearance, colour, odour, texture, consistency and homogeneity. These characteristics were evaated by visual observation. Homogeneity and texture were tested by pressing a small quality of the formulated lotion between the thumb and index finger. The consistency of the formulations and presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. Immediate skin feels (including stiffness, grittiness, and greasiness) was also evaluated.
pH : Lotion pH was measured with a digital pH meter.
10% solution of lotion was prepared and the solution was immersed in the pH meter and the measured pH was recorded.
Irritancy study : Mark an area of 1sq.cm on the left-hand dorsal surface.The lotion was applied to the specified area and time was noted. Irritancy, erythema, edema was checked, if any regular intervals up to 24hrs and reported.
Washability test : A portion of lotion was applied over the skin of hand and allowed to flow under the force of flowing tap water for 10 minutes. The time when the lotion completely removed was noted.
Spreadability : The spreadability of lotion was determined by the parallel plate method. Two glass slides of 20/20 cm were selected. About 1g of the lotion formulation was placed over one of the slides. The other slide was placed upon the top of the lotion such that the lotion was sandwiched between the slides and 125g weight was placed upon the upper slide so that lotion between the two slides as pressed uniformly to form a thin layer. The weight was removed and the spread diameter was measured.
Phase separation : The prepared lotion was transferred in a suitable wide mouth container.
Set aside for storage, the oil phase and aqueous phase separation were visualizing after 24h.
Tube extrudability : In the present study, the method adopted for evaluating formulation for extrudability was based upon the quantity in percentage lotion extruded from tube on application of finger pressure 7. More quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminium collapsible 5 gm tube with a nasal tip of 5 mm opening and applied the pressure on the tube by the help of finger. Tube extrudability was then determined by measuring the amount of lotion extruded through the tip when a pressure was applied on a tube.
Test Microorganism : Bacteria: E.coli, Candida albicans Fungi: A. niger, P.notatum.
Preparation of inoculums : For evaluation of antifungal activity, 24 hours fresh culture of fungi and bacteria were suspended in sterile water to obtain a uniform suspension of microorganism.
Determination of zone of inhibition : Antifungal and Antibacterial activity was checked by agar well diffusion method. In this method a previously liquified medium was inoculated with 0.2 ml of Fungal and Bacterial suspension having a uniform turbidity at temperature of 400C. 20 ml of culture medium was poured into the sterile petri dish having a internal diameter of 8.5 cm. Care was taken for the uniform thickness of the layer of medium in different plates. After complete solidification of liquified inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. In each of these plate test solution was palced carefully. Plates were kept for pre diffusion for 30 mins. After it normalized to room temperature; the plates were incubated at 37ºc for 24 hrs in case of bacteria and at 27ºc for 48 hrs in case of fungi. After incubation period was over, the zone of inhibition was measured with help of Hi-antibiotic zone scale.

RESULTS AND DISCUSSION

Physical Evaluation : The cream is white, appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation.
pH measurement : The pH of the cream was found to 6.2 to 6.7. The pH should not be too acidic as it may cause skin irritation and should not be too alkaline as it may cause scaly skin.
Viscocity measurement : Viscocity was measured by Brookfield viscometer and it was found to be 57310 cps.
Microbiological studies : From the microbial study it was found that the cream showing good effects on microbial growth and the zone of inhibition was calculated by zone reader given in table no. 3.

DISCUSSION

From the above compiled data the study clearly shows that the formulation is showing good in-anti-fungal activity against E.coli and candida albicans. All the extract showed activity against the microbial strains. Neem leaves extract showed excellent activity against both fungus and bacteria. Garlic oil showed more antifungal activity than aloe vera extract. The lotion with mixed extract also showed good activity against both fungi and bacteria. The lotion with increased amount of propylene glycol showed good consistency and with aloe vera it has showed very good moisturizing effect. All the formulations had characteristic odour, Spreadability was observed. The pH required was also in limits .

CONCLUSION

The formulation of antimicrobial agents like *Azadirachta indica* and garlic oil along with aloe vera exhibited enhanced rate of antibacterial and antifungal activity. The results of different chemical and physical tests of lotion showed that it could use topically in order to protect against skin infections caused by fungus or bacteria.

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INGRIDIENTS		W/F WORKING FORMULA (30G)		
	F1(Garlic oil)	F2(Neem extract)	F3(Aloe vera extract)	F4(Herbal mixture)
Herbal Extract	5%	5%	5%	5%
STEARIC ACID	3g	3g	3g	3g
BEESWAX	2.93g	2.93g	2.93g	2.93g
CETYL ALCOHOL	0.363g	0.363g	0.363g	0.363g
PROPYLENE GLYCOL	0.363ml	0.46ml	0.52ml	0.52ml
TRIETHANOLAMINE	0.9ml	0.7ml	0.85ml	0.75ml
LIQUID PARAFFIN	0.6ml	0.6ml	0.6ml	0.6ml
WATER	30ml Qs	30ml Qs	30ml Qs	30ml Qs
PROPYL PARABEN	0.000013	0.000013	0.000013	0.000013

Table 1: FORMULA FOR CREAM DEVELOPMENT

Oil phase	Quantity	Aqueous phase	Quantity
Stearic Acid	2.5%	Propylene glycol	5%
White Bees Wax	1.5%	Triethanolamine	2%
Stearyl Alcohol	5%	Methyl Paraben	0.01%
Cetyl Alcohol	6.5%	Propyl paraben	0.04%
Mineral Oil	5%	Water	Up to 100%
Herbal extract	5%		

Table 2: WORKING FORMULA

Microbial studies bacteria	Zone of inhibition			
	F1	F2	F3	F4
Candida albicans	16mm	20mm	8mm	18mm
E.coli	21mm	25mm	6mm	29mm
A. niger,	25mm	21mm	-	32mm
P.notatum.	22mm	18mm	-	24mm

Table 3: FORMULATED CREAM SHOWING ZONE OF INHIBITION