IJBPAS, January, Special Issue 2, 2022, 11(1): 489-496
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION AND EVALUATION OF POLYHERBAL ANTI ACNE CREAM

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Received 16th June 2021; Revised 11th July 2021; Accepted 25th Aug. 2021; Available online 25th Jan. 2022

https://doi.org/10.31032/ijbpas/2022/11.1.2047

ABSTRACT

Acne vulgaris or acne is the most common skin disease affecting nearly 80% of persons between ages of 11 and 35 years. *Propioni bacterium acnes* and *Staphylococcus epidermidis* are considered as the major skin bacteria that cause the formation of acne. Although acne does not pose serious threat to general health, it is one of the most socially distressing conditions especially for adolescents. In the present study, poly herbal anti acne cream was prepared using extracts of the plants *Mentha pipereta* (Leaves), *Cucurbita pepo* (Seeds) and cade oil along with base materials. The plants have been reported in the literature having good anti- microbial, anti-oxidant and anti-inflammatory activity. Different formulations of the cream were prepared by varying the proportions of materials and evaluated for their physicochemical property like pH, spreadability, viscosity, homogeneity, appearance, and spreadability like tests. Among the different formulations the formulation5 shows better results in evaluation. The main objective is to prepare a cream with natural herbal extracts and minimize the side effects of the chemical cosmetics.

Keywords: Anti acne, Polyherbal cream, *Mentha pipereta* (Leaves), *Cucurbita pepo* (Seeds) and cade oil

INTRODUCTION:

Acne vulgaris or acne is the most common skin disease affecting nearly 80% of persons between ages of 11 and 35 years. Acne affects all races and ethnicities with equal significance.Acne is a common disease of the pilosebaceous units of the skin and acne is an end result of the interplay of multiple factors. Excessive sebum production secondary to sebaceous gland hyperplasia is the first abnormality to occur. Subsequent hyperkeratinization of the hair follicle prevents normal shedding of the follicular keratinocytes, which then obstruct the follicle and form an inapparent microcomedo. The *Propionibacterium acnes*, a resident anaerobic organism, proliferate in the environment created by the mixture of excessive sebum and follicular cells that encourages colonization of *Propionibacterium acnes*, which provokes an immune response through the production of numerous chemotactic and pro-inflammatory factors that may lead to inflammation. Inflammation is further enhanced by follicular rupture and subsequent leakage of lipids, bacteria, and fatty acids into the dermis. The clinical diagnosis of acne is based on the history and a physical examination. Acne most commonly develops in areas with the greatest concentration of sebaceous glands, which include the face, neck, chest, upper arms, and back [1, 2].

As per the guidelines of The American Academy of Dermatology, primary acne vulgaris is classified into mild, moderate and severe grades. Mild acne is characterized by the presence of few to several papules and pustules (without nodules). Patients with moderate acne have too many papules and pustules (along with a few to several nodules) and with severe acne, patients have numerous or extensive papules and pustules (as well as many nodules). Acne is also classified by lesion type as comedonal, papulopustular and nodulocystic [3].

There are many treatments available for Acne vulgaris.Topical therapy is recommended for the management of acne (especially for non-inflammatory comedones and mild to moderate inflammatory acne) and comedolytic, anti-inflammatory agents, alongwith antimicrobials are preferred drugs. In case of comedonal acne, topical retinoids, hormonal therapies or oral isotretinoin are mostly available treatment. Benzoyl peroxide, topical antibiotics such as Clindamycin, Erythromycin, Tetracyclin and oral Isotretinoin or combination of all these medications are available for mild to moderate inflammatory acne. During the past

few decades, many reports have documented an emergence of antibiotic resistance by *Propionibacterium acnes* during treatment of acne. Furthermore, systemic antimicrobial usage has been causally associated with various short-term and long-term adverse events.

To address the above problems of Acne vulgaris, major research activities have been directed towards developing an acne control composition that can be effective against acne [4-6].

The present work basically aims to identify a novel herbal remedy that possess anti inflammatory, anti kerolytic, sebum control and anti bacterial agent and methods of developing the same as cosmeceutical product for topical application for control and treatment of acnevulgaris.

MATERIALS AND METHOD

All the chemicals used in this investigation of analytical reagent (AR) grade. Distilled water was used for complete study. All glassware's and equipments used for the handling of bacterial cultures and plant extract were sterilized prior to use. Sterilization procedures were performed by autoclaving at 121^0c for 15 minutes.

Selection and Collection of Drugs

The following plants were selected for the present study; the selection was done on the basis of literature survey, *Mentha pipereta* (Leaves), *Cucurbita pepo* (Seeds) and cade oil.

Preparation of Extracts

The shade dried material of leaves of *Mentha pipereta* and seeds of *Cucurbita pepo* was pulverized to coarse powder.500gms of the drugs were defatted by petroleum ether and then subjected to Soxhlet extraction for 4 hrs with methanol. The solvent was removed using rotary evaporator to get dry residue. 100mg of each extract was weighed individually and dissolved in 10mL water.

Antibacterial Activity of Extracts

The methanolic extracts of the selected plants and oil were subjected to preliminary antibacterialscreening using cup plate method.

The stock solutions of the plant extracts were prepared in double distilled water as per the solvent specifications. The working solution (100 mg/ml) was prepared accordingly.

The method for antibacterial activity is based on diffusion of antibacterial compound from the reservoir hole to the surrounding inoculated nutrient agar medium such that the growth of the microorganism is inhibited as a circular zone around the bore.

All the standard cultures were obtained from NCL Pune.

The organisms were maintained by subculturing at regular intervals in nutrient agar medium.

Methodology:

Media used: nutrient agar (NA) was used as base medium for screening of antibacterial activity andnutrient broth (NB) for preparation of inoculums.

Preparation and standardization of inoculums: Four to five colonies from pure growth of each test organism were transferred to 4-5 ml of NB. The broth was incubated at 35-37^{o}C for 18-24 hours. The standardized inoculums suspension was inoculated within 15-20 minutes.

Technique used-Well Diffusion: Screening of antibacterial activity was performed by well diffusion technique. The NA plates were seeded with0.1 mL of the standardized inoculums of each test organism. The inoculums were spread evenly over plate with loop or sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37^{o}C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the NA and about 100 µL of each formulation was introduced in the well.

Incubation: The inoculated plates were incubated at 35-37^{o}C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm).

Formulation of Herbal cream [7-10]

Excipients used in the cream formulation

Stearic acid, Potassium hydroxide was procured from Qualigens Fine Chemicals and Glycerine, Methyl paraben, Propyl paraben were procuredfrom Ranbaxy Fine Chemicals Limited.

Preparation of cream

Cream was prepared by using the extracts of *Mentha pipereta* (Leaves), *Cucurbita pepo* (Seeds) and cade oil and taking different proportions of excipients as shown in **Table 1**. Accurately weighed amount of stearic acid was taken and kept on water bath at 80^{o}C. All the methanolic extracts were weighed and dissolved in water which was also kept at same temperature. Potassium hydroxide was also added in to the extracts solution. All the oils were dissolved in melted stearic acid. The aqueous extract solution was added slowly in to the stearic acid with stirring and allowed the mixture to cool. The prepared cream was filled in aluminum tubes and the ends were sealed by crimping the ends.

Table 1: Formulation of polyherbal cream

S. No.	Ingredients	Formula %w/w				
		F1	F2	F3	F4	F5
1	Extract of *Mentha pipereta*	1	1.5	1.5	2	2.5
2	Extract of *Cucurbita pepo*	2.5	1	2.5	2	2.5
3	Cade oil	1.5	1.5	1.5	1.5	1.5
4	Methyl Paraben	0.018	0.02	0.02	0.018	0.02
5	Propyl Paraben	0.02	0.02	0.02	0.02	0.02
6	Potassium hydroxide	2	2	2	2	2
7	Glycerine	10	1	12	10	12
8	Stearic acid	10	15	20	11	12
9	Distilled water	QS	QS	QS	QS	QS

Evaluation of the Formulation [11-14]

The herbal dermatological formulation was prepared using cream base incorporating all the necessary ingredients along with the extracts. Formulation was then evaluated for its physical property,viz. pH, extrudability and antimicrobial activity, antiacne property.

pH of the Cream: The pH meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.

Determination of Spreadability:

Spreadability denotes the extent of area to which the dermatological formulation readily spreads on application to skin or the affected part. The bioavailability efficiency of a formulation also depends on its spreading value. The Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the Spreadability. Two sets of glass slides of standard dimensions were taken. The herbal cream formulation was placed over one of the slides. The other slide was placed on the top of the formulation, such that the cream was sandwiched between the two slides in an area occupied by a distance of 6.0 cm along the slide. 100gm weight was placed upon the upper slides so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times and the mean time taken for calculation.

Spreadability was calculated by using the following formula:

$$S = m \times \frac{l}{t}$$

Where, S – Spreadability, m – Weight tied to the upper slide (20gm), l – Length of the glass (6 cm), t – Time taken in seconds.

Determination of Extrudability:

It is a useful empirical test to measure the force required to extrude the material from a tube. Since the packing of creams and gels have gained a considerable importance in delivery of desired quantity of gel from a jar or extrusion of gel from collapsible tube, therefore measurement of extrudability becomes an important criterion.

The formulation were filled in standard caped collapsible lami-tube and sealed. The tube was weighed and recorded. The tube was placed between two glass slides and was clamped. A 500gm weight was placed over the glass slide and then cap was opened. The amount of cream extruded were collected and weighed. The percent of gel extruded was calculated; and grades were allotted (++++ Excellent, +++Good, ++ Fair, +Poor).

Anti-acne Activity of formulation

The antibacterial activity of differentformulations was determined by modified agar welldiffusion method. In this method, nutrient agar plateswere seeded with 0.2 ml of 24 h broth culture of *P.acnes*. The plates were allowed to dry for 1 h. Asterile 8 mm borer was used to cut four wells ofequidistance in each of plates; 0.5 ml of solutions offormulations, extracts, tea tree oil, Aloe vera gel, marketed herbal formulation and tetracycline wereintroduced in to the wells at randomly. The plates wereincubated at 37^0C for 24 hours. The antibacterialactivity was evaluated by measuring the diameter ofzones of inhibition (in mm). The experiments repeatedfour times.

RESULTS

The results of this investigation showed that all developed formulations had inhibitory effect on the *P.acnes*. Formulation 5 has higher activity than that ofother developed formulations. The activity of thedeveloped formulation 5 has been comparable to thatof marketed preparation. However, the activity of thestandard tetracycline was more than that of all developed formulations, marketed herbal anti-acne preparation, extracts, *Mentha pipereta* (Leaves), *Cucurbita pepo* (Seeds) and cade oil. The diameterof zones of inhibitions is given in **Table 2**.

Table 2: Preliminary evaluation of prepared cream formulations

Formulation	pH	Extrudability	Spreadability	Antiacne property (mm)
F1	**6.1**	++	**124**	**6**
F2	**6.4**	++	**137**	**7**
F3	**6.3**	++	**148**	**7**
F4	**6.3**	+++	**161**	**8**
F5	**6.5**	++++	**166**	**8**

DISCUSSION

Amongst all the formulations F5 had very optimum Spreadability and good antiacne property with 8mm zone of inhibition compared with the standard drug as of 10mm. All the formulationsshowed considerable zone of microbial inhibition. Preliminary characterization of cream has shown that pH of formulation was approachingtowards neutrality and variations in cream base composition significant effect on pH of the formulation. Acne vulgaris is an extremely common skindisorder that affects virtually all individuals at leastonce during life. The incidence of acne peaks atteenage, but substantial numbers of men and womenbetween 20-40 years of age are also affected by thedisorder. Acne can have important negativepsychosocial consequences for the affected individual, including diminished self-esteem, social withdrawaldue to embarrassment and depression.Herbal medication are considered safer thanallopathic medicines as allopathic medicines areassociated with side effects such as like contactallergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, xerosis of the skin etc. The formulations having antibacterial agents inhibiting the *P.acnes*, may also reduce the development of inflammatory acne.

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