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Research Article

Formulation of Topical Gel from Extract of *Berberis aristata* DC for Acne

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ABSTRACT
In current study, an attempt has been taken to formulate Topical gel of ethanolic extract of *Berberis aristata* DC for antiacne activity. The gel containing extract was prepared by adding gel-forming material in sterile distilled water while the mixture was stirred and allowed to stand to hydrate. Extract was added in PEG 400 and polyethylene glycol mixture. Methyl paraben and propyl paraben were added as the preservative and triethanolamine was added as the neutralizer. The anti-acne activity was investigated against two causative bacteria, i.e., *Propionibacterium acnes* and *Staphylococcus epidermidis* and yeast (*Malassezia furfur)* by the well diffusion method. The result showed that gel passes all test of evaluation and was found to be active against acne causing microorganisms. Extract based gel has potential activity against acne-causing microorganism.

Keywords: Indian barberry, Daruhaldi, , Herbal gel, *Berberis aristata* DC , *Propionibacterium. Acnes*

INTRODUCTION

Acne is an exclusive disease associated with skin occurs when sebaceous glands attain special conditions at face, chest and back at prepubertal child. After the onset of puberty androgen production increases within the body and sebaceous glands are the main target organ for the same because they have higher androgen receptor density in the human skin. *P. acnes* predominantly found in sebaceous gland rich area of the skin in adults. In the human skin it exists from the birth till the death of the person. The pathogenicity of *P. acnes* is thought to be because of *P. acnes* resistance towards phagocytosis and they can persist inside the macrophages for prolong period of time. Sebum production is directly associated with the growth of *P. acnes*. One of the hypothesis also said that in response to the invading microorganism the Phagocytes of the body such as neutrophils release reactive oxygen species in order to cause lyses of the invading cell. These reactive oxygen species are involved in irritation and destruction of follicular wall[1,2].

Current treatment of acne consists of use of topical or systemic antibiotics, retinoids etc. There is a great concern about the increase incidence of antibiotic resistance and its uncommon side effects. Oral isotretinoin is believed to cause teratogenic effects apart from other side effects like mucocutaneous symptoms[3].

To overcome the problem associated with allopathic medicines i.e. contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, xerosis of skin, antibiotic resistance etc, herbal formulations are proposed to make, as medicinal plants have anti-bacterial, anti-inflammatory, anti-oxidant and anti-androgen activities and devoid of all other adverse effects.

There is a need to develop different therapeutic options for the treatment of acne. Herbal medicine is very effective in curing various dermatological diseases and there are many herbal drugs which have been mentioned to be useful in the treatment of acne which shows marvelous results. But the problem with these treatments is the method of application in which they are used.

Medicinal plants could be a choice due to its efficacy and devoid of side effect[4]. In skin diseases medicaments are mainly applied topically. Herbal medicine is very effective in curing various dermatological diseases and there are many herbal drugs which have been mentioned to be useful in the treatment of acne. One of them is *Berberis aristata* DC (BA). "In our previous research it was found that , ethanolic extract of *Berberis aristata* (EEBA) could be choice of alternative for curing acne"[5]. In this context, EEBA has been screened for formulation of topical gel for the aforesaid anti-acne activity.

MATERIALS AND METHODS

Materials

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Table 1: Various prepared formulation of EEBA

Ingredients	Quantity taken per 100 g (in grams)											
	I						II				III	
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
B. aristata extract	0.5	0.5	1	1	1	1	1	1	1.5	2	1.5	2
Carbopol 934	1	1.5	1	1.5	1.5	1.5	2	2	2	1.5	1.5	2
PEG 400	20	20	20	20	20	30	20	30	20	20	20	20
Propylene glycol	10	10	10	10	10	10	10	10	10	10	10	10
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Purified water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Development of formulation of anti-acne gel from EEBA
(q.s – Quantity sufficient)

Carbopol 934 (Qualikems Pvt. Ltd., Mumbai, India), PEG 400 (Loba Chemie, Mumbai, India), propylene glycol), methyl paraben(Central Drug House Pvt. Ltd., New Delhi) , propyl paraben (Central Drug House Pvt. Ltd., New Delhi), triethanolamine (Titan Biotech Ltd., Rajasthan, India), methanol (Jiangsu Huaxi International Trade Company Ltd., China), Berberin (sigma Aldrich) , BHI agar (HiMedia Pvt. Ltd., Laboratories, Mumbai, India) , MH agar (HiMedia Pvt. Ltd., Laboratories, Mumbai, India), PDA (HiMedia Pvt. Ltd., Laboratories, Mumbai, India) etc.

Methods

Plant materials and extract preparation

Dried stem of BA were procured from the local commercial suppliers of Jalandhar, Punjab. Authentication of BA was done in Department of Botanical and Environmental Sciences, GNDU, Amritsar, Punjab, and the voucher specimens have been deposited at the school of pharmaceutical sciences, Lovely professional University. The crude plant material was pulverized in coarse powder form for the purpose of extraction. Coarsely powdered dried plant drug material was extracted by Soxhlet's apparatus using alcohol as solvent[6].

Collection of microbial strains

Staphylococcus epidermidis (Aerobic bacteria, MTCC 3382), *Propionibacterium acnes* (Anaerobic bacteria MTCC 1951) and fungal strain *Malassezia furfur* (MTCC 1765) were collected from the "Microbial Type Culture Collection Centre", "Institute of Microbial Technology", Chandigarh.

Preparation of anti-acne gel

The gel containing EEBA was prepared by adding gel-forming material in sterile distilled water while the mixture was stirred and allowed to stand to hydrate. Extract was added in PEG 400 and polyethylene glycol mixture. Methyl paraben and propyl paraben were added as the preservative and triethanolamine was added as the neutralizer[6]. Various prepared formulation are shown in table 1

Methodology for development of formulation

I The first 2^2 factorial design was taken care for optimization. Two variables were the content of Carbopol 934 and EEBA. The amount of Carbopol 934 were 1% and 1.5% and those of EEBA were 0.5% and 1%. Different formulation were prepared (F1-F4).

II The second 2^2 factorial design taken care for optimization included the content of Carbopol 934 and Polyethylene glycol 400. The concentrations of Carbopol 934 were 1.5% and 2% and that of PEG 400 were 20% and 30%. Four formulas of anti-acne gel were prepared (F5 – F8).

III The third 2^2 factorial designs included two variables i.e. one was concentration of plant extracts and other was concentration of carbopol polymer. The concentrations of Carbopol 934 were 1.5% and 2% and that of plant extracts were 1.5% and 2% (F9 – F12).

Evaluation of gel

The various gel formulations were developed containing the EEBA at different concentrations followed by their evaluation considering different parameters such as homogeneity, pH, viscosity and spreadability in order to select the best out of many prepared gels. The selected gels were to be further evaluated for their release studies.

pH measurements

pH of the gels were determined by a digital pH meter by dipping the glass electrode entirely in to the formulation to cover the electrode[7].

Homogeneity

Visual inspection in presence of light techniques was adopted for ensuring homogeneity of gel[7].

Viscosity

Brookfield viscometer (Spindle type, S-62) at 10 rpm was used to determine viscosity of gel. The spindle of brookfield viscometer was dipped beaker containing 100g of gel. Run the instrument for 5 minutes and reading was noted in cps[7].

Spreadability

The gel was transferred on a glass slide and covered with an equivalent slide. The slides are placed in such a way that gel sandwiched up to 7.5 cm. A weight of 50g was placed over upper slide which helped in forming uniform thin layer. The weight was removed and excess adhering gel was wiped. After that 20 g weight was coupled carefully to upper slide. Time taken to travel a distance of 7.5 cm by upper slide under influence of weight was recorded. The procedure was repeated thrice and the mean was calculated. The following formula was used to determine spreadability.

Fig. 1. (a)

Fig. 1. (b)

Fig.1. Preparation of gels (a) 1% gel of *B. aristata* (b) 2% gel of *B. aristate*

Fig.2. Photographs of the developed formulations after filling in tubes

S = MxL/T

Where, S - Spreadability

M - Weight coupled to the upper slide (50 g)

L - Length of the glass slide (7.5 cm)

T- Time taken to separate the slides in seconds[7].

Extrudability

20 g of gel was filled in a closed collapsible tube and pressed safely at the crimped end. A clamp was used to check rollback. The cap was separated and the gel was extruded until the pressure was dissipated. A good quality gel extrude from collapsed tube with slight pressure applied.

In-vitro skin permeation studies

Franz diffusion cell were used for In vitro skin permeation studies with a receptor compartment capacity of 25 ml and an effective diffusion area of 2.54 cm2. The cellophane membrane of required thickness was hydrated 24 hours before use with pH 7.2 phosphate buffer saline. The donor compartment contained 1 g gel and recipient compartment consisted of phosphate buffer saline pH 7.2. At regular interval sampling was done from receptor compartment and equal amount of fresh phosphate buffer saline was added in the receptor compartment. The active constituent present in the samples withdrawn was determined by UV spectroscopy[8,9].

Antiacne assay of different formulations

Table 2: -Physical evaluations of developed gel formulations

	Formulation of *Berberis aristata* extract											
Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Appearance	++	++	+++	+	+++	++	++	++	+	+++	++	+
pH	6.2	6.4	6.7	6.5	6.93	6.27	6.6	6.48	6.03	6.93	7.1	8.02
Viscosity	21869	47823	23227	34784	29186	65125	46157	53180	32174	27467	48246	61212
Spreadability	13.184	27.296	14.646	18.472	16.98	37.902	23.857	25.428	17.298	19.143	24.076	33.176
Gelling	++	+	+++	-	++	+	++	+	++	+++	++	-

+++ Excellent, ++ Good, + Average

The antiacne activity of selected formulations was determined by disc diffusion method. In this method, MH and BHI agar plates were seeded with 24 hr broth culture of S. epidermidis and P. acnes also PDA plates were seeded with 7 days broth culture of M. furfur. A sterilized borer was used to make wells in each of plates; 100µg of gel and erythromycin disc (15µg) were introduced into the plates along with 100µg of marketed erythromycin gel (2% w/w). The plates were incubated at 37°C for 24 hours in case of S. epidermidis and for 24-48 hours in case of P. acnes and M. furfur. Diameter of zones of inhibition (in mm) was measured for evaluation of anti acne activity[10].

Stability study

Accelerated stability study was conducted for optimized gels at 40°C and 75% RH for one month using Navyug India Ltd. stability chamber[7].

Table 3:- Anti acne activity of selected formulation

Microorganism	Zone of inhibition	
	1% B	2% B
P. acnes	13.03±0.12	14.17±0.03
S. epidermidis	8.23±0.12	10.13±0.07
M. furfur	14.03±0.03	17.93±0.03

RESULTS AND DISCUSSION

Preparation of gels

All the developed gels were examined visually for appearance, nature and homogeneity matching the desired characteristics of gel and the following gels were selected.

Evaluation of gels

The various gel formulations were optimized on the basis of different physical parameters. On the basis of the results obtained of all the batches, the best formulations meeting the characteristics of gel were selected.

It was observed that F3 and F10 batches comply with the desired characteristics of gel and was selected for further evaluation of antimicrobial assay.

Antimicrobial assay of selected formulations

The antibacterial activity of developed formulations was evaluated by measuring the diameter of zones of inhibition (in mm). The results of this investigation showed that the selected formulations F3 and F10 of BA showed inhibitory effect on the test bacterium which is comparable to marketed preparation. However, the activity of the standard erythromycin gel was more than that of all developed formulations. The zones of inhibition are given in table 3.

On the other hand, the activity of selected formulations against M. furfur showed remarkable inhibitory effect as compared to standard drug i.e. fluconazole. Comparative study of antiacne activity of formulations as separate entity and in combination against *P. acnes*, *S. epidermidis* and *M. furfur*.

In-vitro skin permeation studies for developed gels

Drug diffusion study of the herbal gel was essential to confirm that the extract would partition from the vehicle and permeate through the semi permeable membrane which symbolizes the stratum corneum. The study was carried out for a period of 6 hours in which the gel demonstrated % Cumulative drug release as depicted in table 4.

Stability study

Stability study which was conducted for optimized batches stored at 40°C/75%RH for one month do not shows any stability issues after evaluation (appearance, feel on application, pH, viscosity.

CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. So, herbal anti-acne solution which is non-toxic, safe, effective, and improves patient compliance by the utilization of herbal extracts would be highly acceptable. The present study was undertaken with an aim to formulate and evaluate gel formulation of ethanolic extract of stems of *B. aristata*. The study involves anti

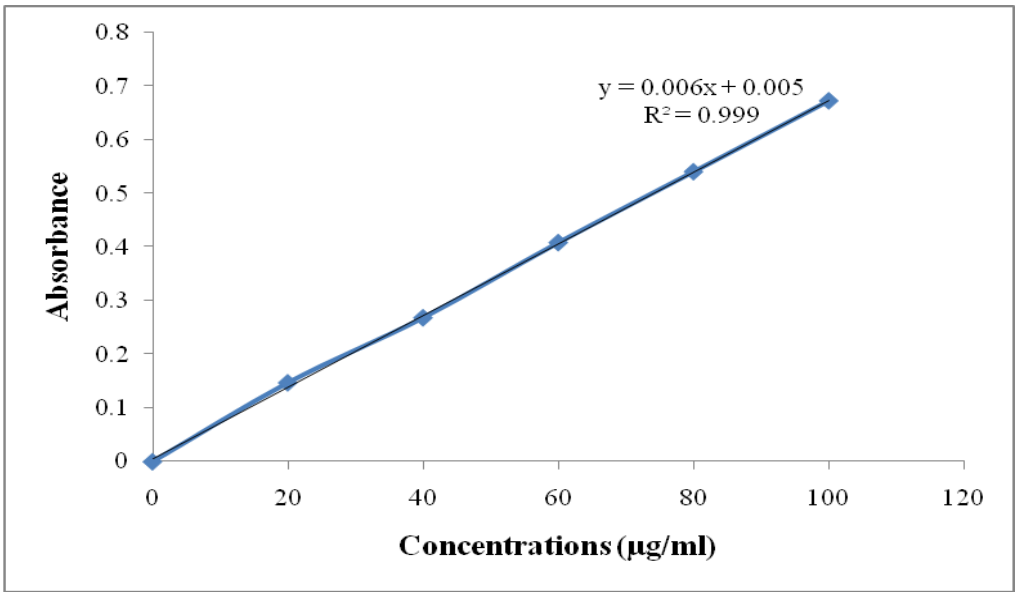

Fig.3 Standard plot of berberine hydrochloride)

Table 4 - Diffusion study of developed gels of plant extracts

Time (min)	% Cumulative release of gels % Cumulative release of Berberine	
0	1% B	2% B
10	0	0
20	8.83	10.86
30	17.27	24.75
40	23.21	28.01
50	30.14	34.63
60	39.37	41.35
90	47.42	45.92
120	50.85	52.55
180	56.47	65.94
240	59.03	69.57
300	60.78	70.88
360	61.14	70.74

acne activity of gel, however an extensive study is required to prove the activity clinically.

The plant extracts are proven to be active against *P. acnes*, *S. epidermidis* and *M. furfur* which are responsible for the outbreak of acne. The results of the antimicrobial studies obtained for the prepared extracts of *B. aristata* reflect the potential for treatment of acne vulgaris which forms the basis of our study. Thus, the present study proved to be significant from the point of view that gels of extracts have been developed and evaluated for anti acne activity. The prepared gels comply with the pharmacopoeial standards, followed by stability study which was conducted for optimized batches stored at 40°C/75%RH for one month. Gels were evaluated after one month for different parameters such as appearance, feel on application, pH, and viscosity. It was concluded that the above selected formulations were stable[8].

Thus, the present research work suggests that the prepared herbal extract based gel formulations holds good potential against acne and can prove to be efficacious remedy for treating this dermatological disorder with added advantage over the currently used antibiotic treatment in the fact that the bacteria which often develop tolerance and resistance to the antibiotics over time may not be seen here.

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