Research Article

Development and Evaluation of Herbal Cream of *Berberis aristata* for its Antibacterial and Antifungal Activity

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Abstract

In the present study, an attempt was made to formulate and evaluate herbal cream of *Berberis aristata (*B. *aristata*) for the management of Psoriasis. The study is intended to be carried out because of low cost, lesser or no side effects of herbal formulations and its potent action than the allopathic medication. They therefore provide a viable alternative for psoriasis management. The drug was evaluated on the basis of U.V, FT-IR, and DSC study. The herbal cream formulations were prepared by emulsion based method. The prepared cream was evaluated on the basis of viscosity, spreadability, grittiness, and in-vitro drug release. With altering the ratio of base different release patterns of the drug were observed. With an increase in the base ratio, there was increase in drug release of formulation (F1), while further increase in the base ratio leads to initial burst release but as the time proceeds the release of the drug got decreased due to increase in grittiness and viscosity of the formulation (F2). Also decrease in the base ratio leads to decrease in the drug release of formulation F3. The FT-IR and DSC analysis indicates that there was no interaction between drug and excipients. The in vitro drug release kinetics study depict that drug release mechanism follows Fickian diffusion. The optimized F1 formulation which shows satisfactory in vitro release was tested for anti bacterial and anti fungal property. The zone of inhibition was found to be 24.66 ± 1.14 and 20.45 ± 1.26 for bacteria and fungi respectively at 250 ppm.

Keywords: *Berberis aristata*; Herbal Cream; Emulsion Based Method; Antifungal; Antibacterial Activity; Skin Irritancy Test and Release Models

Introduction

Psoriasis is a very common, non-infectious, inflammatory skin disease characterized by well defined, distinctive erythematous plaques yielding adherent silvery white scales, which may manifest bleeding points when removed (Auspitz's sign) [1,2]. Psoriasis may affect any cutaneous surface, but the commonest sites are the extensor surfaces of the elbow sand knees, scalp (where scales may become extremely dense) and sacral areas. Precipitation of psoriasis may occur due to trauma (scratching, sunburn or surgical wounds), Kobner phenomenon, stress or genetic predisposition. Men and women are equally affected, but it is more common in white people than in Asians and black people [1].Psoriasis is either benign and localized (hands and feet) or generalized or life threatening, with associated fever, leucocytosis, arthralgias, diffuse cutaneous and mucosal pustules, secondary infection and electrolyte disturbances [3].

Herbal products have been applied to human healthcare for long-established time. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health [4].

Berberis aristata, (family-*Berberidaceae)* plant is intended to be used in the herbal formulations because of its potent anti psoriatic action (anti inflammatory) due to the presence of active compound berberine. It shows minimum risk of side effects and having great potential for health management. *B. aristata* contains isoquinoline alkaloids mainly berberine given in figure 1. Other phytoconstituents includes oxyberberine, berbamine, aromaline, kakrachine, palmatine, oxycanthine, taxilamine, and jatrorrhizine, some of the alkaloids are reported as being found in the chloride salt form [5].
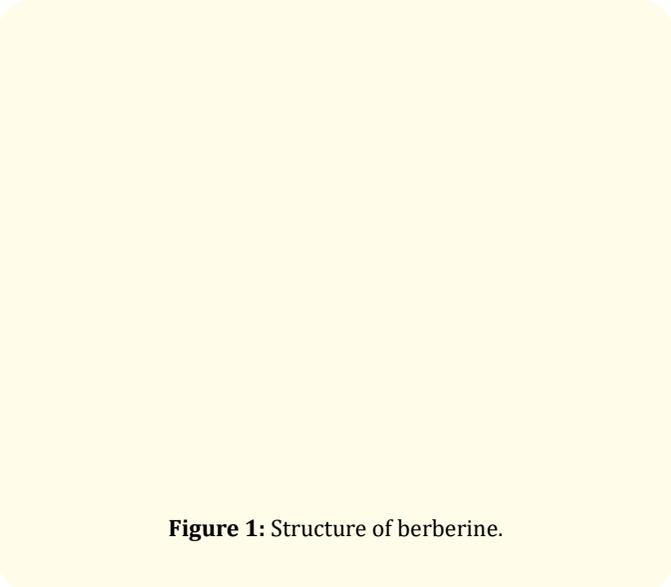
Figure 1: Structure of berberine.

Materials and Method

- Berberine standard, *Berberis aristata* extract, Neem seed oil and Camphor was obtained as gift sample from Herbal bioactive lab Jamia Hamdard, New Delhi, India.

- The micro-organisms *Bacillus subtilis* and *Candida albicans* was obtained from Microbial cell laboratory and gene bank, Chandigarh, India.

- Stearic acid, Isopropyl myristate, Cetyl alcohol, Triethanolamine, Glycerine, Methyl paraben, Propyl paraben, Salicylic acid, Zinc oxide and Butylated hydroxyl anisole was obtained from Standard chemicals, New Delhi, India.

- All chemicals and reagents used were of analytical grade.

Standardization of *Berberis aristata*

The evaluation of a crude drug involves the determination of identity, purity and quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine [6]. Various standardization parameters were evaluated to obtain the qualitative information about the purity and quality of *Berberis aristata* such as total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, foreign matter, melting point, loss on drying [7].

Identification tests

Identification tests such as Ultraviolet (UV) spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, and Differential Scanning Colorimetry (DSC) were conducted.

U.V. spectroscopy method
Preparation of UV plot of berberine in 6.8 phosphate buffer solution

Berberine (from *Berberis aristata* extract) serial dilutions were made. Initially a solution of the concentration of 1000 mcg/ml was made by weighing 10 mg of drug using digital balance (Shimadzu, AU X 220) and dissolving in 10 ml of 6.8 phosphate buffer solution. From this stock solution different concentration ranging from 10 - 100 mcg/ml were made in and absorbance of the resulted solution were taken on the PC using 6.8 phosphate buffer solution as blank. The detection wavelength was set at 350nm for the detection of Berberine [8]. A graph was plotted between the concentration (X-axis) and absorbance (Y-axis).

FT-IR method

FT-IR was obtained using Shimadzu FT-IR spectrophotometer, model iraffinity-1CE. The infra red spectrum of the standard Berberine and drug sample (*Berberis aristata* extract) was recorded in the range of 800-4000 cm^{-1} using potassium bromide pellet method and compared for any interactions present [9,10].

Differential scanning calorimetry (DSC)

DSC of the pure drug was taken by using differential scanning calorimeter [11] (Perkin Elmer DSC-7) calibrated with Indium. All samples were run in triplicate. The instrument was adjusted to the following parameters:

- Atmosphere: Nitrogen inert.

- Heating rate: 10ºC/min

- Gas flow rate: 20 ml/min

- Temperature range: 30 - 200ºC

- Sample size: 0.5 mg.

Formulation development of anti-psoriatic cream

Oil in water (O/W) cream (semisolid formulation) was formulated by emulsion-based method. The oil soluble components (Cetyl alcohol, stearic acid, isopropyl myristate, neem seed oil, camphor, salicylic acid, and BHA) were dissolved in the oil phase and heated to 75°C. The emulsifier (triethanolamine), preservatives (methyl paraben and propyl paraben), and other water soluble components (*B. aristata* extract and glycerine) were dissolved in the aqueous phase and heated to 75°C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifer took place [12]. Then zinc oxide is added slowly to the semisolid with continuous stirring until it is properly blended in it. The formula for the cream is given in table 1.

Each 100 gm contains.			
Ingredients	**F1 (gm)**	**F2 (gm)**	**F3 (gm)**
B. aristata extract	20	20	20
Stearic acid	18	20	16
Isopropyl myristate	2	3	1
Cetyl alcohol	4	4.5	3.5
Triethanolamine	2	3	1
Glycerine	1	1.5	0.5
Methyl paraben	0.95	1.4	0.5
Propyl paraben	0.095	1	0.05
Neem seed oil	5	7	3
Camphor	5	6	4
Salicylic acid	2	2.5	1.5
Zinc oxide	2	2.5	1.5
BHA	1	1.5	0.5
Water	q.s.	q.s.	q.s.

Table 1: Batch design with varying the concentration of base.p

Evaluation parameters of cream

- **Viscosity:** Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

- **pH:** 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 [13] within 24hrs after formulation.

- **Homogeneity:** The formulations were tested for the homogeneity by visual appearance and by touch.

- **Spread ability:** It was determined by wooden block and glass slide apparatus [14]. For the determination of spread ability, excess of sample was applied in between two glass slides and they were compressed to form a uniformly thick layer of cream. The upper slide was then pulled apart horizontally with a string and pulley system. Initially 10 gm weight was tied to the thread and left for 5 minutes, and then the weight was increased by 1 gm at every step. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of Spread ability (S).

Spread ability was calculated by using the formula:

S = ML/T, Where,

S = Spread ability

M = Weight tied to upper slide

L = Length moved on the glass slide

T = Time taken to separate the slide completely from each other.

- **After feel:** Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

- **Type of smear:** After application of cream, the type of film or smear formed on the skin was checked.

- **Removal:** The ease of removal of the cream applied was examined by washing the applied part with tap water.

In-Vitro drug release study

The phosphate buffer with a pH of 6.8 was used as a receptor medium for the *in-vitro* drug release studies. The membrane was soaked in phosphate buffer pH 6.8 for 6 - 8h and was clamped carefully to diffusion cell. The formulated cream (5g) was accurately weighed and placed in the donor part of the Franz diffusion cell and a semi permeable cellulose membrane with a molecular cut off point of 1000. The cell body was filled with degassed phosphate buffer, pH = 6.8. The receptor phase was stirred thoroughly by a constantly spinning bar magnate at 100rpm. Intervals of 0.5, 1, 2, 4, 6, 8 and 12 hrs were chosen for sampling time [14]. The sample of 5 ml each was withdrawn at predetermined time interval and were replenished immediately with the same volume of phosphate buffer maintaining sink condition throughout the experiment and samples were analyzed for drug content spectrophotometrically at 350 nm.

Release kinetics

The cumulative drug release obtained from the optimized formulation was used for the calculation of release kinetics i.e. zero order, first order, Higuchi's square root of time equation plot, and Korsmeyer-peppas' power law equation model [14]. All the models are compared for the best fitting of the model.

Anti fungal and anti bacterial screening

Disc diffusion technique was selected for antifungal and antibacterial activity study. These tests were carried out using cultures of *Bacillus subtilis* [15,16] and *Candida albicans* [16] in Sabouraud-dextrose agar media. An amount of 15 ml (each) of the media with 24-hour subcultures of *Bacillus subtilis* and *C. albicans* and was distributed in each petridish (10-cm diameters) respectively and allowed to solidify. On solidification, microbial suspension was spread with the help of sterilized cotton swab on the surface of the

media. The filter paper disc was prepared from Whatmann's filter paper, which then impregnated with formulation having berberine onto the surface of agar plates, over which a culture of the microorganisms has been streaked. Zone of inhibition diameter (mm) were then recorded [17]. The study was carried out in an aseptic area. The experiment was performed in sterilized condition.

Skin irritation test

The irritancy of the optimized formulation was determined in the albino Wistar rats procured from R.V. Northland animal house facility (Reg. No. 1149/PO/Ere/S/07/CPCSEA). About 1g cream was applied to the left ear of the albino Wistar rat and the right was considered as a control [18,19]. The development of erythema and oedema were monitored for 3 days using the reported method [20].

Results and Discussion

Properties	Observed value	Reported value [according to LAB. monograph]
Appearance	Yellowish brown powder	Yellowish brown powder
Taste and odour	Bitter with aromatic, characteristic odour	Bitter with aromatic, characteristic odour
Melting point	151.213°C	145°C
Loss on drying at 105°C	10.5%	*NMT 25%
Total ash	15.78%	*NMT 28%
Acid insoluble ash	7.75%	*NMT = 15%
Alcohol soluble extractive	6.3%	**NLT = 4%
Water soluble extractive	36.8%	**NLT = 32%
Foreign matter	0.68%	*NMT = 1%

Table 2: Physico-Chemical Properties of *Berberis aristata* extract.

*NMT: Not More Than; **NLT: Not Less Than.

As shown by the table 2, the physico-chemical properties of the drug was found in accordance with that of standard monograph.

Ultraviolet spectroscopy

UV plot of berberine in pH 6.8 phosphate buffer solution.

The calibration curve of U. V. depicts that it obeys Beer's Lambert law where, slope = 0.0045 and R^2 = 0.9975.

Figure 2: Calibration curve of Berberine through U.V.

Figure 3: FT-IR Spectra of Berberine in *B. aristata* extract.

Figure 4: FT-IR spectra of standard Berberine.

Fourier-transform infrared spectroscopy

Wave number (cm^{-1})	Characteristic functional group feasible	Compound type	Standard detected at (cm^{-1})	Sample detected at (cm^{-1})
2850-2500 cm^{-1}	Ar C-H stretch	AROMATIC	2848	2848.98
900-600 cm^{-1}	Ar C↓H	AROMATIC	839	839.07
1650-1535 cm^{-1}	C=C stretch	Cyclo alkenes	1600	1601.95
1690-1520 cm^{-1}	C=N stretch	Heterocyclic amines	1681	1633.78
1330-1050 cm^{-1}	C-O stretch	Lactone	1065	1059.93

Table 3: Comparative FT-IR spectra of Berberine extract with standard berberine.

Interpretation of FT-IR

FT-IR interpretation peaks of Aromatic C-H stretch, Aromatic C↓H, C=C stretch, C=N stretch and C-O stretch of the sample was found nearly equal to that of standard as shown by the table 3. Thus, interaction studies depicts that the drug sample was shown to be fairly stable and inert to that of standard used in the formulation.

Differential scanning colorimetry of *B. aristata* extract

The melting point of the extract was found to be 151.213°C which is in accordance with the standard monograph (145°C) of berberine which confirms the presence of Berberine in the extract of *B. aristata*.

Parameters	Formulation		
	F1	F2	F3
pH	6.80	5.89	5.84
Homogeneity	+++	++	++
Spread ability	28.86	27.27	25.45
Viscosity (cps)	6800	7000	7200
After Feel	E	E	E
Type of smear	NG	NG	NG
Removal	ES	ES	ES

Table 4: Evaluation data of formulation F1, F2, F3 for various parameters.

+++: Good; ++: Satisfactory; E: Emollient; NG: Non Greasy; ES: Easy.

Time (hrs.)	%CPDR		
	F1	F2	F3
0.5	30.5 %	25%	23.5%
1	40.5%	35.5%	30.1%
2	49.5%	46.5%	36.5%
4	57.5%	54.99%	43.5%
6	68.5%	59%	50.5%
8	71.5%	60%	51.5%
12	76.5%	60.5%	55.5%

Table 5: Cumulative percent drug release of F1, F2 and F3.

Figure 5: DSC analysis of berberine in *B. aristata* extract.

As shown in the table 4, there is emolliency and non greasiness in the above formulations F1, F2 and F3 and they are easy in removal. F1 formulation shows pH similar to that of skin, good homogeneity and high spread ability as compared to formulations F2 and F3. The low viscosity of F1 depicts that the cream is easily spreadable by small amount of shear.

In-vitro drug release study

Drug release from the formulations F1, F2, and F3 shows that F1 formulation has maximum drug release i.e. 76.5% at 12th hour

Figure 6: Drug release profile of formulation F1, F2, F3.

in comparison with the F2 and F3 in which drug release is 60.5% and 55.5% respectively.
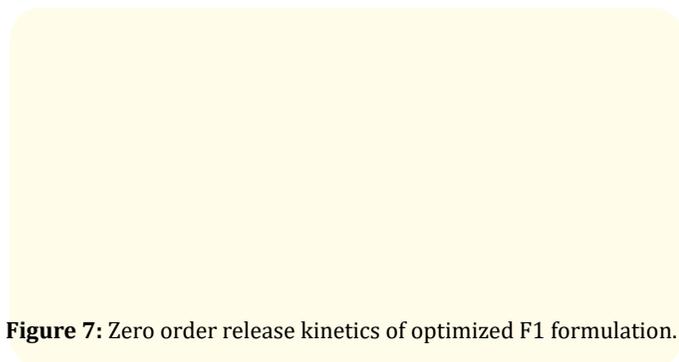
In-vitro release kinetics study

Figure 7: Zero order release kinetics of optimized F1 formulation.
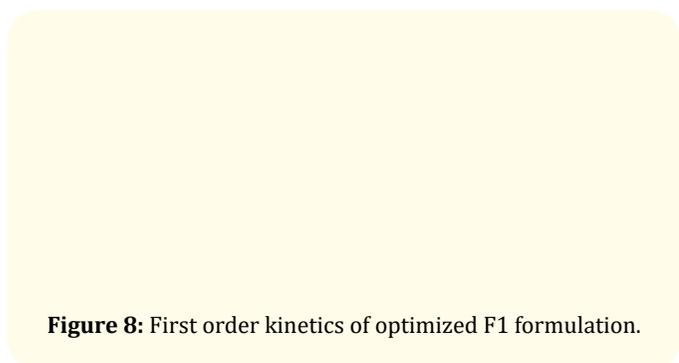

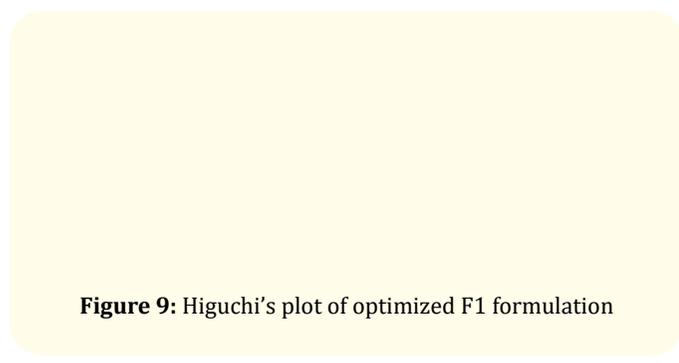**Figure 8:** First order kinetics of optimized F1 formulation.
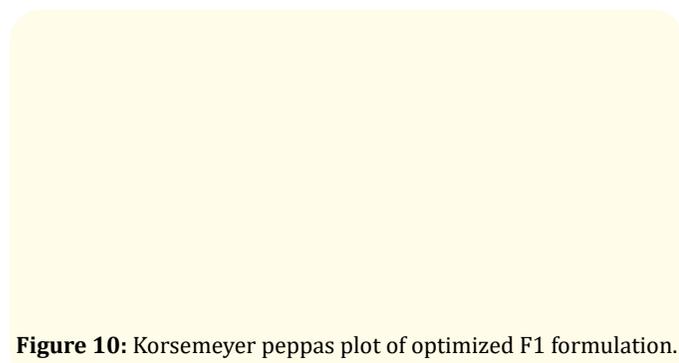

Figure 9: Higuchi's plot of optimized F1 formulation

Figure 10: Korsemeyer peppas plot of optimized F1 formulation.

Release Model	R^2	SLOPE=K
Zero order model	0.8613	0.7582
First order model	0.9377	-0.0932
Higuchi's square root of time plot	0.9601	3.3355
Korsemeyer peppas plot	0.9534	0.2912

Table 6: Model fitting of the drug release profile of formulation F1.

Interpretation of release models (In Vitro)

The formulation so proposed is following first order release kinetics (first order release model, R^2= 0.9377), where drug is being released through diffusion process (Higuchi model R^2 = 0.9601). The cream follows Fickian diffusion (n = 0.2912 from Korsmeyer-Peppas law equation).

Anti fungal and anti bacterial screening data

Category	Test organism	Diameter of zone of inhibition (in mm)			
		Formulation			
		250ppm/disc		500ppm/disc	
		Cream	*Control	Cream	*Control
Bacteria	*Bacillus subtilis*	24.66 ± 1.14	6.69 ± 0.59	26.26 ± 1.23	6.69 ± 0.59
Fungi	*Candida albicans*	20.45 ± 1.26	6.14 ± 0.61	22.62 ± 1.28	6.14 ± 0.61

Table 7: Zone of inhibition of the optimized formulation (F1).

*Control: Methanolic extract of *B. aristata*.

From the above mentioned result and by comparing the zone of inhibition values of the test (cream) with the control, it is observed that optimized formulation (F1) has larger zone of inhibition i.e. 24.66 ± 1.14 and 26.26 ± 1.23 for bacteria and 20.45 ± 1.26 and 22.62 ± 1.28 for fungi at 250 ppm/disc and 500 ppm/disc respectively. So it may be concluded that the optimized formulation F1 exhibits better antibacterial and antifungal activity.

Skin irritation test data

The skin irritation test was performed to confirm the safety of the herbal formulation. Aqil., *et al.* [21] mentioned that a value of skin irritancy score between 0 and 2 indicates that the applied formulation is non-irritant and safe for human skin. The mean value of skin irritancy score for formulation was found to be 1.16 (Table 8). This value indicates that all excipients used in formulation were safe for topical drug delivery.

Skin irritation scores of formulation (A = Erythema formation score; B = Oedema formation score)							
Rats	Intact skin				Abraded skin			
	24 hours		72 hours		24 hours		72 hours	
	A	B	A	B	A	B	A	B
1	0	1	0	1	0	1	1	0
2	0	0	1	0	0	1	0	1
3	1	1	0	1	0	2	1	1
Final skin irritation scores of formulation (* = Total of A and B from part a.; **= Average of all skin reading of 24 and 72 hours)							
Rats	Intact skin		Abraded skin		Total Average			
	24 hours 72 hours		24 hours 72 hours		(i)+(ii)			
	(i)		(ii)					
1	1	1	1	1	1			
2	0	1	1	1	0.75			
3	2	1	2	2	1.75			
					Combined avg.=1.16			

Table 8: Scores of formulation (F1).

Conclusion

From the result of present study, it can be concluded that F1 formulation was optimized from all the batches. FT-IR study revealed that there is no possible drug interaction with other components present in extract and DSC study depicts that berberine is present in the extract. An *in vitro* drug release study showsF1 releases the drug up to 76.5% in 12 hour. Release mechanism follows first order kinetics where drug is being released through diffusion process (Higuchi model R^2 = 0.9601). The formulation follows Fickian diffusion (n = 0.2912 from Korsmeyer-Peppas law equation). Also the formulation is non irritant and can be applied easily. Thus, Berberine based anti-psoriatic cream has great potential for the management of psoriasis.

Acknowledgements

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