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Research Article

FORMULATION AND EVALUATION OF ANTIACNE CREAM CONTAINING *WITHANIA SOMNIFERA*

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ABSTRACT
Acne vulgaris is a disease of pilosebaceous unit which is characterized by non-inflammatory and inflammatory lesions. Common therapies that are used for the treatment of acne include topical, systemic, hormonal, herbal and combination therapy. So, for the treatment of acne herbal anti acne cream is prepared containing *Withania somnifera* extract, which is known for its anti-inflammatory action and antioxidant property. Herbal medications are considered safer than allopathic medicines because allopathic medications are associated with different side-effects such as allergy, local irritation, scaling, photosensitivity reaction, itching, peeling, redness etc. The study includes macroscopical and microscopical evaluation of *Withania somnifera* containing ash value, loss on drying, water extractive value, Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry and Ultraviolet Spectroscopy. The prepared formulation was optimized on the basis of *in vitro* release study. The optimized formulation was evaluated on the basis of greasiness, spreadability, after feel, skin irritancy, viscosity, pH and release kinetics. Anti bacterial study by disc diffusion technique was performed. The result obtained was zone of inhibition of test 25.66 ± 1.12 and 5.89 ± 1.18 at 500 ppm, this proved that optimized formulation has possible anti acne property. So, further investigation such as *in vivo* studies will help this formulation to be a potential candidate as marketed product.
Keywords: *Withania somnifera*, Acne vulgaris, anti acne cream, FTIR, skin feel test.

INTRODUCTION

Skin is the most vulnerable part of our body. This is well known fact that day to day exposure to sun, dust leads to number of problems such as pimples, acne, sunburn marks and pigmentation.[1,2] Acne vulgaris is a disease of pilosebaceous unit which is characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules and nodes). The conditions usually start at the age of 14 to 19 years. The word acne is derived from "acme" which means "prime of life". A change in keratinisation pattern of hair follicle leads to blockage of sebum secretion.[3,4] It is hypersensitivity to the stimulation of sebocytes and follicular keratinocytes by androgen leads to hyperplasia of sebaceous glands and seborrhea which characterize acne. Acne can be classified according to predominance of specific skin lesions:[3]

- Comedonal (non inflammatory) mild
- Papular (inflammatory) mild to moderate
- Pustular (inflammatory) moderate
- Nodulocystic severe

The most common bacteria responsible for acne is *Staphylococcus aureus* but some studies report that *Propionibacterium acnes* have also been isolated from acne patients. The *P. acnes* colonize the follicular duct and proliferates, which results in the conversion of sebum into triglycerides which are probably responsible for development of inflammation. The use of natural remedies as well as herbal medication is in practice since thousand years. With minor side-effects and much advantages of multi functionality, the herbal medicines are being used in different formulation to cure many diseases.[5] The aim of the study is:

- Formulation development of anti acne cream
- Evaluation of anti acne cream

MATERIALS AND METHOD

Plant Materials

Roots of Asgand nagori (*Withania somnifera)*, Roghan Aneesi (oil), Kushta zast (zinc oxide bhasm), Roghan Laung (oil) were obtained as sample from Hamdared (WAKF) Laboratories. The microorganism *Propionibacterium acne* obtained from microbial cell laboratory and gene bank, Chandigarh, India.

Chemicals

All chemicals used were of analytical grade and is obtained from standard chemicals, New Delhi, India.

Apparatus

Fourier transform Infra red Spectroscopy, Ultraviolet Visible Spectrophotometer, Differential scanning calorimeter, Dissolution Apparatus USP I.

Physico chemical analysis of *Withania somnifera*

Colour: buff to grey-yellow
Odour: characteristic
Taste: bitter and acrid[6,7]

Total Ash Value

Heat silica crucible to red heat for 30 minutes, allow cooling and weighing it. Unless otherwise specified in the individual monograph weigh accurately about 1 g substance under examination and evenly distribute it in crucible. Dry at 100o to 105o for 1 h and ignite to
Constant weight. Allow to cool after each ignition. The material should not catch fire at any time during procedure. If

after prolong ignition carbon free ash cannot be obtained as directed in method. Calculate the % wt of ash on dried basis.[8]

Acid Insoluble Ash

Boil the ash (Total ash method) with 25 ml of hydrochloric acid for 5 minutes, collect the insoluble matter in ash less filter paper (Whatmann Filter paper), wash with hot water, ignite, cool and weigh. Calculate the percentage of acid insoluble ash on dried basis[8].

Moisture content (LOD)

5 g of powder were weighed accurately in petridish and kept in hot air oven maintained at 110^0C for 4 h. After cooling in dessicator loss in weight was recorded after every hour. The procedure was repeated till constant weight was obtained.[8]

Analytical Methodology of *Withania somnifera* Extract in Phosphate Buffer

UV Spectroscopy

Stock solution of powdered drug was prepared by dissolving 10 g of drug in phosphate buffer (6.8pH). Required dilutions of 1 ppm, 2 ppm, etc were prepared. The prepared dilutions were scanned in the UV region of 225 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR was obtained using a Shimadzu FTIR spectrophotometer, model Iraffinity- 1CE. In order to collect the spectra powdered drug was taken and its KBr pellets were prepared. It was analyzed from 500 to 4000 cm^{-1} range[9].

Differential Scanning Calorimetry

DSC of the pure drug was taken by using differential scanning calorimeter (Perkin- Elmer DSC- 7) calibrated with indium. All samples were run in triplicate. The instrument was adjusted to the following parameters:[10]

- Atmosphere: Nitrogen inert.
- Heating rate: 10ºC/min
- Gas flow rate: 20 ml/min
- Temperature range: 0-300ºC
- Sample size: 0.5 mg

Formulation Development of Cream Base

Oil phase and water phase were taken in separate beakers and heated upto70^0C. Oil phase was added in water phase with continuous stirring till oil in water emulsion was prepared.[11,12]

Formulation Development of Anti acne Cream

In small amount of base sulphurated potash was added and mixed well using mortar and pestle, to it roghan aneesi and roghan laung was added. In other part of base asgand extract was mixed and to it zinc oxide and kushta zast were added and khushboo rose were added and mixed well. Both part were mixed together and glycerine was added and triturated well.[11,12]

Table 1: Formulation of anti acne cream

Ingredients	F1	F2	F3
Withania somnifera extract	10 %	10 %	10 %
Sulphurated potash	3 %	3 %	3 %
Roghan Aneesi	0.04 %	0.04 %	0.04 %
Roghan laung	0.04 %	0.04 %	0.04 %
Kushta zast	3 %	3 %	3 %
Zinc oxide	5 %	5 %	5 %
Khushboo rose	1.25 %	1.25 %	1.25 %
Stearic acid	0.9 %	0.7 %	1.1 %
Cetyl alcohol	16.22 %	16.22 %	16.22 %
Tri ethanolamine	0.51 %	0.51 %	0.51 %
Isopropylmyristate	0.95 %	1 %	0.90 %
Methyl paraben	0.095 %	0.095 %	0.095 %
Glycerine	0.9 %	1.15 %	0.65 %
Water	58 %	57.99 %	58.19 %

Evaluation Parameter of Cream

pH

About 20 mg of the formulation was taken in beaker and subjected to ph measurement using digital pH meter.[13]

Viscosity

Viscosity of the formulated cream was determined using Brookfield viscometer spindle # 7 at 25^0C. The dial reading was multiplied by the factor mentioned.[13]

Spreadability

500 mg of the cream was sandwiched between two slides. A weight of 100 g was applied on the upper slide. The weight was removed and extra formulation was scrapped off. The lower slide was fixed on board of apparatus and upper slide was fixed with non flexible string on which 20 g load was applied. Time taken by the upper slide to slip off was noted down.[13]

$$\text{Spreadability} = m \times l/t,$$

m = weight tied on upper slide, l = length of glass and t = time in seconds

After feel

After application of fixed amount of cream emmoliency and greasiness was checked

***In vitro* Drug Release**

The dissolution study were performed in six station dissolution apparatus (37 ± 0.5°c, 50 rpm) using USP paddle type method in phosphate buffer solution (pH 6.8,900 ml) for next 24 h. The sample of 5 ml each was withdrawn at predetermined time interval and replenished immediately with the same volume of phosphate buffer maintaining sink condition throughout the experiment. The aliquots were analyzed spectrophotometrically at 225 nm. The concentration of drug in test samples was calculated using regression equation.[14,15]

Release Kinetics

The cumulative drug release obtained from the formulation was used to obtain release kinetics i.e. Zero order, First order, Higuchi square root of time plot and Koysmear-peppas power law model.[14,15]

Skin Irritation Study

The irritation study was performed over the skin of albino rats. 1 g of cream was applied on left ear of the albino rat and right ear was considered as control. The development of erythema and oedema was observed for 3 days according to the reported method.[16,17]

Antibacterial Property

Disc diffusion technique was selected for anti microbial study. The test was carried out using *Propionibacterium acne* Sabouraud-dextrose agar media. An amount of 15 ml of the media with 24 h subculture of *Propionibacterium acne* was distributed in each petridish and allowed to solidify. On solidification microbial suspension was spread with the help of sterilized cotton swab on the surface of media. The filter paper disc was prepared with Whattman's filter paper, which then impregnated with drug *Withania somnifera* present in anti acne cream and control (extract of *Withania somnifera*) on to the surface of agar plates on which culture of microorganisms has been streaked. Zone of inhibition diameter was then recorded for test and control (28^{0}C after 24 h) and compared. The study was carried out in aseptic area. The experiment was performed in sterilized condition.[13,16]

RESULTS

Table 2: Physicochemical analysis of Drug

Parameters	Observed value	Reported value (Acc. To Hamdard monograph)
Loss on drying	6.6 %	NMT 12 % w/w
Foreign matter	Nil	NMT 1 % w/w
Total ash	6.36 %	NMT 7 % w/w
Acid insoluble ash	0.37 %	NMT 1 % w/ww
Water soluble extractive	18.59 %	NLT 15 % w/w
Melting point	246^{0}C	245^{0}C- 249^{0}C

*NMT= not more than, **NLT= not less than

As shown in the table above, physicochemical properties of the drug were calculated. It was found to be in accordance with the standard values of the Hamdard Monograph.

Evaluation of Cream

pH

pH of the formulation was found to be 6.9 ± 0.5.

Viscosity

Viscosity of the formulation was measured after 24 h of the formulation and was found to be 33.5 ± 0.3 cps.

Spreadability

Spreadability of the cream was found to be 40.8 ± 0.5 g/sec.

After Feel Effect

Cream was found to be of emollient nature and non greasy.

UV Spectroscopy

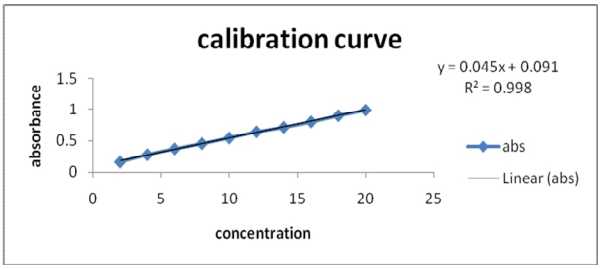

Figure 1: Calibration plot of *Withania somnifera* in Phosphate buffer pH 6.8

Standard graph was obtained on plotting the concentration on x axis and absorbance on y axis with $r^2 = 0.998$

Fourier Transform Infrared Spectroscopy Study

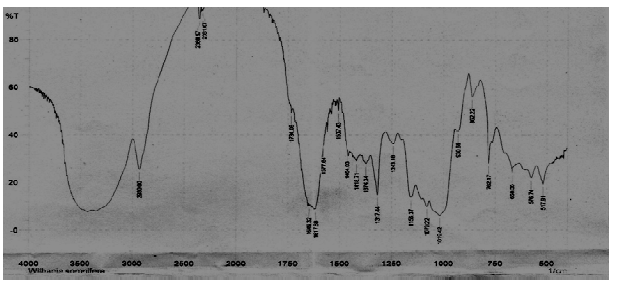

Figure 2: FTIR spectrum of *Withania somnifera*

Interpretation of FT-IR

The FT-IR analysis of the samples was done and the functional groups associated were determined (Figure 2). The FT-IR spectrum of the *Withania somnifera* samples recorded the number of peaks lying between 2990 cm^{-1} – 2500 cm^{-1}, 1700 cm-^{1} – 1440 cm^{-1}, 1243 cm-^{1} – 1019 cm^{-1}, 862 cm^{-1} - 517.19 cm^{-1} which corresponds with the standard value.

Differential Scanning Calorimetry (DSC)

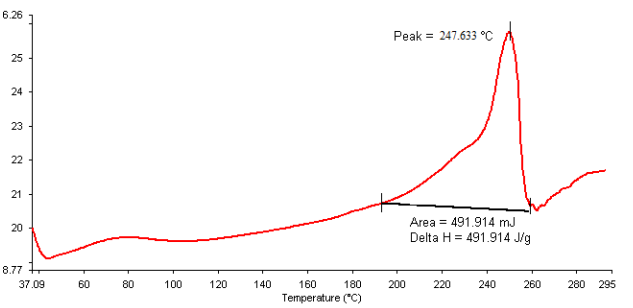

Figure 3: DSC curve of withanolide

The melting point of extract was found to be 247.633^{0}C which is found to be in accordance with the melting point of withanolide i.e. 245^0 C – 249^{0}C. This shows that withanolide is present in the extract of *Withania somnifera.*

Drug Release Profile

Table 3: Cumulative percentage drug release of Formulation F1, F2 and F3

Time	% CPDR		
	F1	F2	F3
0.5	19.76	13.76	11.76
1	28.56	21.76	17.73
1.5	32.94	28.56	24.56
2	43.16	34.76	28.56
4	57.76	42.56	35.9
6	64	45.15	44.16
12	66.56	54.7	50.16
24	72.1	55.7	54.7

% CPDR: Cummulative Percentage Drug Release

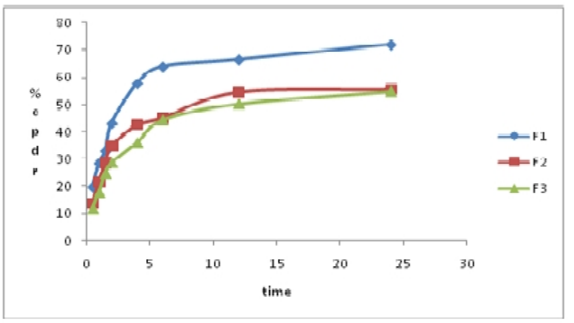

Figure 4: Drug release of F1, F2, F3

Drug release from F_1, F_2 and F_3 shows that F_1 has maximum drug release of 72.1 % as compared to F_2 and F_3 which has drug release of 55 % and 54 % respectively. So the optimized formulation F1 shows highest drug release obtained on the basis of release is F1.

In vitro Drug Release Kinetics

Figure 5: First Order Release Model

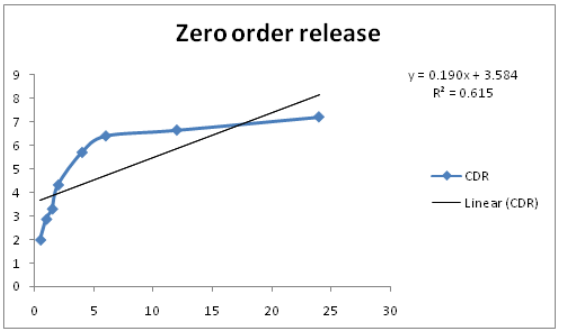

Figure 6: Zero Order Release Model

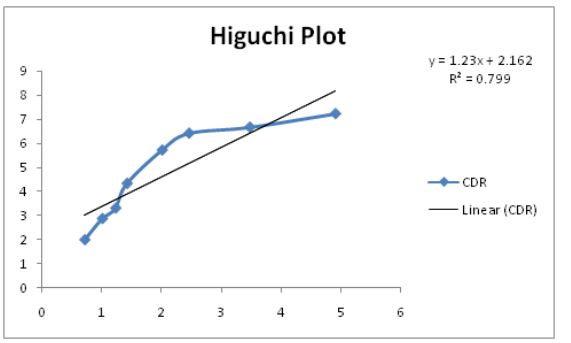

Figure 7: Higuchi Plot

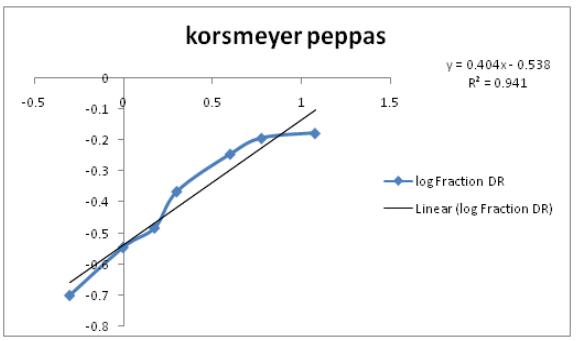

Figure 8: Korsmeyer Peppas Model

Table 4: Release Kinetics Data

Release model	R^2	Slope = k
Zero order release	0.615	0.190
First order release	0.967	4.852
Higuchi plot	0.799	1.23
Korsmeyer –peppas model	0.941	0.404

Interpretation of Release Model (*In vitro*)

The optimized formulation F1 follows first order release kinetics (r^2 = 0.967) i.e. drug is being released through the diffusion process. The formulation follows Fickian diffusion (n = 0.404, as obtained from Korsmeyer – peppas law equation)

Skin Irritancy Study

Table 5: Scores of Optimized Formulation

A: skin irritation scores of anti acne cream AC1(A*= erythema score formation B** = oedema score formation)								
	Intact skin				Abraded skin			
Rats	24 h		72 h		24 h		72 h	
	A*	B**	A*	B**	A*	B**	A*	B**
1	1	1	1	0	1	0	1	0
2	2	0	1	1	0	1	1	0
3	1	1	2	0	1	1	0	1

B: final skin irritation scores of anti acne cream AC1 (*= total of A and B from part A and ** = average of all skin reading from 24 and 72 h)

	Intact skin		Abraded skin		Total average of i and ii
	24 h and 72 h		24 h and 72 h		
Rats	(i)		(ii)		
1	2*	1*	1*	1*	1.25**
2	2	2	1	1	1.5**
3	2	2	2	1	1.75
Combined average					1.5

The test was performed to confirm the safety of the cream. It is mentioned that score between 0 and 2 indicates that formulation is non irritant and safe for human skin. The mean value of skin irritancy was found to be 1.5. This indicates that excipients used in the formulation are safe and are non irritant for the human skin and can be used for topical delivery.

Anti acne Property of Cream

Table 6: Zone of inhibition of sample and control

Formulations	Zone of inhibition (mm)	
	500 ppm/disc	250 ppm/disc
Cream	25.66 ± 1.12	22.34 ± 1.03
Control	5.89 ± 1.18	5.59 ± 0.05

Control = extract of *Withania somnifera*

Anti acne property of cream was evaluated using disc diffusion technique. Zone of Inhibition values for the test and control were observed. The observed average ± SD values for test and control at 500 ppm/disc are 25.66 ± 1.12 and 5.89 ± 1.18, at 250 ppm/disc is 22.34 ± 1.03 and 5.59 ± 0.05 respectively. It was found that test has significantly large zone of inhibition compared to control. Thus, it possess better anti acne property.

CONCLUSION

Anti acne cream was formulated using *Withania somnifera* extract known for its anti-inflammatory and antioxidant property. Three formulations were prepared and were optimized on the basis of *in-vitro* release study. Drug was standardized on the basis of different parameters such as UV spectroscopy, FTIR and DSC study. The optimized formulation was evaluated on the basis of skin irritancy test, after feel and anti acne property. Thus the result shows that above formulation can be used effectively for the treatment of acne vulgaris.

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