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FORMULATION AND EVALUATION OF HERBAL CREAM OF EUCALYPTUS GLOBULUS AND ALOE BARBIDENSE LEAVES EXTRACT FOR ANTI-ACNE ACTIVITY

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ABSTRACT

The present research work deals with the formulation and evaluation of herbal cream against acne vulgaris. The herbals are having the worldwide market now- a-days. The ethanolic extracts of eucalyptus (leaves) and aloevera (leaves) were formulated into a cream. The plants are having good anti- microbial, and Anti inflammatory according to the literature survey. Seven batches of o/w herbal cream was formulated and named as F1-F7. These formulations are evaluated according to physicochemical properties and also the study includes microscopical and macroscopical evaluations. The prepared formulation was optimized by in vitro release study.The cream,s effectiveness is compared to that of a commercially available acne treatment product.

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INTRODUCTION

Acne vulgaris is a chronic inflammatory skin disorder of the pilosebaceous unit that affects the face, back, and trunk, which contain the greatest oil glands[1.] The main micro-organisms responsible to cause the condition include Propioni bacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis[2.] Indian herbs and its significance are popular in worldwide. Herbal cosmetics are in high demand around the world and are a priceless gift from nature. When compared to synthetic medications, herbal formulations have always received a lot of attention because of their potency and lack of adverse effects. These days the helpfulness of spices in the cosmeceutical creation has been widely expanded in private consideration framework and there is an incredible interest for the natural beauty care products[3.]

In the present study two south Indian herbs are used, they are Eucalyptus globulus and Aloe barbidensis plants[4] efficacious and cheap anti-acne drugs with least side effects. Therefore these two drugs were selected for study. Main work is aimed to design and develop herbal anti acne cream .

MATERIALS AND METHODS

Eucallyptus globulus and Aloevera was collected from the medicinal garden of Hindu College Of Pharmacy, Stearic acid, Cetyle alcohol, Glycerol, Methyl paraben and Propyl paraben was purchased from Loba Chemise laboratory reagents and fine chemicals Ltd, Mumbai, India, Paraffin oil and Triethanol amine was Purchased from Qualigens, Agar-Agar powder, Potato dextrose Agar, Propylene glycol and Ethanol was purchased from SD Fine chemicals.

Plant material and extract preparation[5]:

Eucallyptus globulus and Aloevera was collected from the medicinal garden of Hindu College Of Pharmacy, Guntur, Andhra Pradesh, and both the plants were authenticated by Dr. P. Satyanarayana Raju, Plant taxonomist, Department of Botany and Microbiology, ANU, Guntur. The foreign earthy matter and residual matters were removed carefully from the leaves, cleaned and dried in shade. The dried leaves were powdered and used for extraction. Here we are doing maceration extraction process by using ethanol as a polar solvent. Maceration involved soaking of plant materials (powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of 7 days. Then the extract was filtered and concentrated using Rota-vaccum evaporater. It was stored in a cool or dark place until use.

FORMULATION DEVELOPMENT[6-8]:

The composition of Eucalyptus globulus and Aloe barbidense mill herbal cream formulations was shown in the table 5.3. Different formulations were prepared using different ratios of concentrations of both extracts.

The formulation can be prepared by adding two phases which are mentioned as following

Phase 1: The emulsifying agent stearic acid was dissolved in cetyle alcoho and heated to70º c. It can be named as oil phase i.e., Part A.

Phase 2 In this phase mix the both above collected extracts of aqueous and alcoholic followed by adding preservatives other water soluble components ike methyle paraben, propyle paraben, triethanolamine and heated to 70˚c. It can be named as aqueous phase i.e., Part B.

After heating aqueous phase which contains the extracts was added into oil phase at same temperature with continuous stirring the smooth & homogenous cream was prepared. After cooling perfume was added.

CHARACTERIZATION OF CREAM:

Physical Examination:

The pre arranged cream definitions are examined outwardly for their shading, appearance and extrudability, and stage partition.

pH Evaluation test:

pH assessment is the significant rules particularly for the effective definition. The pH of the cream ought to be between 5-7 copy the skin condition. Assuming the pH of the pre-arranged cream is acidic or fundamental, it might make aggravation the patient. pH of the prepared cream was measured using digital pH meter (ELICO L1 613).1gm of cream was dissolved in 100ml of distilled water and it was placed for 2hr and then dip the glass electrode into an cream.The estimation of pH of every formulation was done in three fold and normal values were determined.

Spreadability:

Spreadability denotes the extent of area to which the cream readily spreads on application to skin or the affected part. The bioavailability efficiency of an cream 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 which was 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 cream, such that the cream was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slide.

100gms 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 cream 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 of freely by the force of weight tied to it. A 10 gm weight was tied to the upper slide carefully. The time taken for the upperslide to travel the distance of 6.8 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was separated by three times and the mean time was taken for calculation and spreadability was calculated using formula:

S=M.L/T

Where M=weight tied to upper slide (10gm)
L=length of glass slide (6.8cm)
T= Time taken to separate the slides.

Drug content determination[9]:

1 gm each formulation containing approximately 40 mg of drug was taken in a 50 ml volumetric flask and diluted with ethanol and shaken to dissolve the drug in ethanol. The solution was filtered through whatmann filter paper. 0.1 ml of the filtrate was pipetted out and diluted to 10 ml with ethanol. The content of the drug was estimated spectrophotometrically by using standard curve plotted at 276 nm.

Drug content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor.

***In-vitro* Release/Permeation studies[10]:**

In-vitro release studies were carried out using Franz diffusion cell of 10ml capacity. Egg membrane was isolated and used for the study. Pre-weighed cream was spread evenly on the egg membrane. The egg membrane was clamped between donor and receptor compartment. The receptor compartment was filled with 10 ml of pH 6.8 phosphate buffer maintained at 370C and stirred by using magnetic stirrer. 1ml sample was collected at suitable time intervals (i.e., for every 30mins until complete drug was released) and replaced with fresh buffer. The collected samples were analyzed for drug content by UV- Visible spectrophotometer at 276nm for Eucalyptus and 220nm for Aloevera.

***In-vivo* Studies Of Optimized formulation:**

Study protocol:

Study period : 7 days
Plant part used for study : Dried leaves of Eucalyptus and Aloevera
Induction model proposed for study: Skin irritation study
Experimental studies : Rats(wistar albino)

No. of animals required for study : 18

Experimental design:

In the experiment, a total of 18 rats were used. The rats were divided into 3 groups comprising of 6 animals in each group as follows:

GROUP 1: Control (Glycerin)
GROUP 2: Standard Drug (Clindamycin 10µg/ml, topical)
GROUP 3: Herbal cream formulation (10 mg/kg, topical)

Skin Irritant Test[11]:

0.5gm of the optimized formulation was applied on the hair free skin of rabbits by uniform spreading an area of 4cm^2. The skin surface was observed for any visible change such as erythema (redness) after 24, 48 and 72 hours of applying the formulation. The mean erythmal sores are recorded depending on the degree of erythema.

No erythema = 0
Slight erythema (barely perceptible-light pink) =1
Moderate erythema (dark pink) = 2
Moderate to severe erythema (light red) =3
Severe erythema (extreme redness) = 4

Stability studies[12-18]:

Stability studies were carried out for the optimized formulation according to international conference on Harmonization (ICH) guidelines. Short term accelerated stability studies were carried out for the period of 3 months for the formulations. The samples were stored at different temperature conditions i.e., refrigeration temperature (4-8ºC), room temperature (25±2ºC) and oven maintained at (45±2ºC). The stored formulation was analyzed for visual appearance, clarity, pH, spreadability, viscosity and drug content.

RESULTS AND DISCUSSION

Compatability studies:

From the FT-IR spetra of physical mixture of the drug, excipients, and other ingredients, it was revealed that there was no chemical interactions of plants extracts and other ingredients.

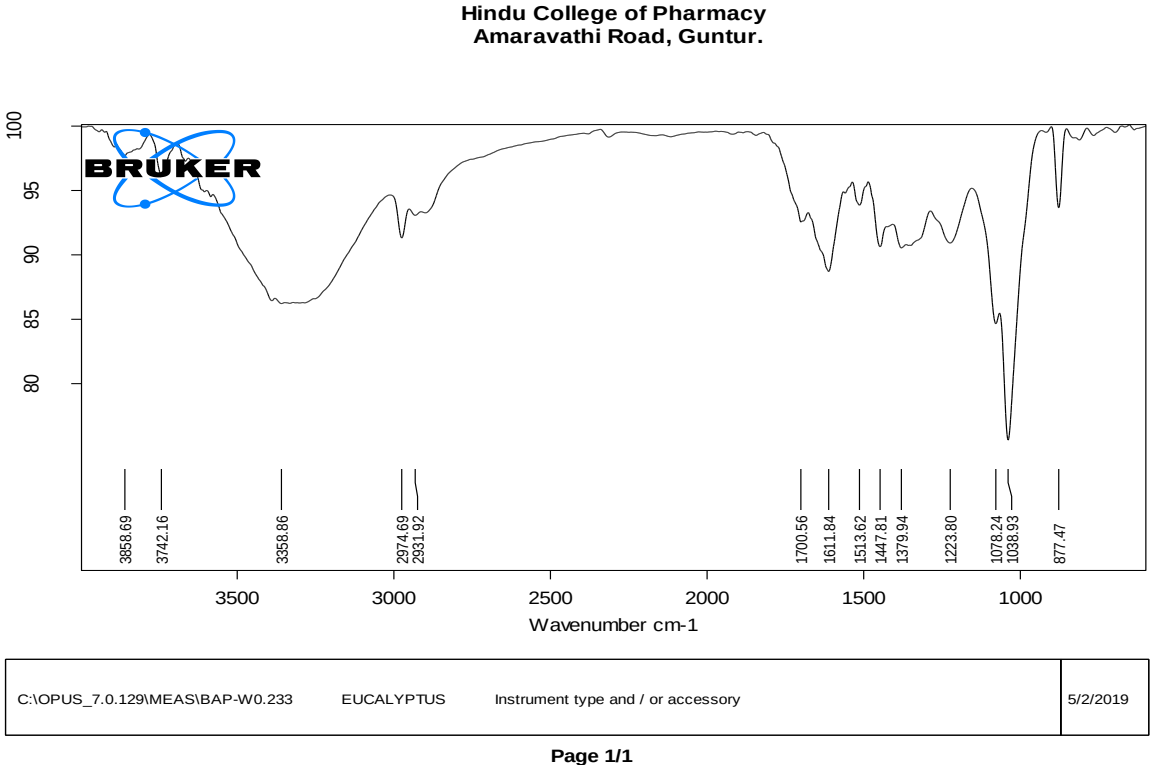


Fig 1 : FT-IR Spectrum of etanolic extract of Eucalyptus globules.

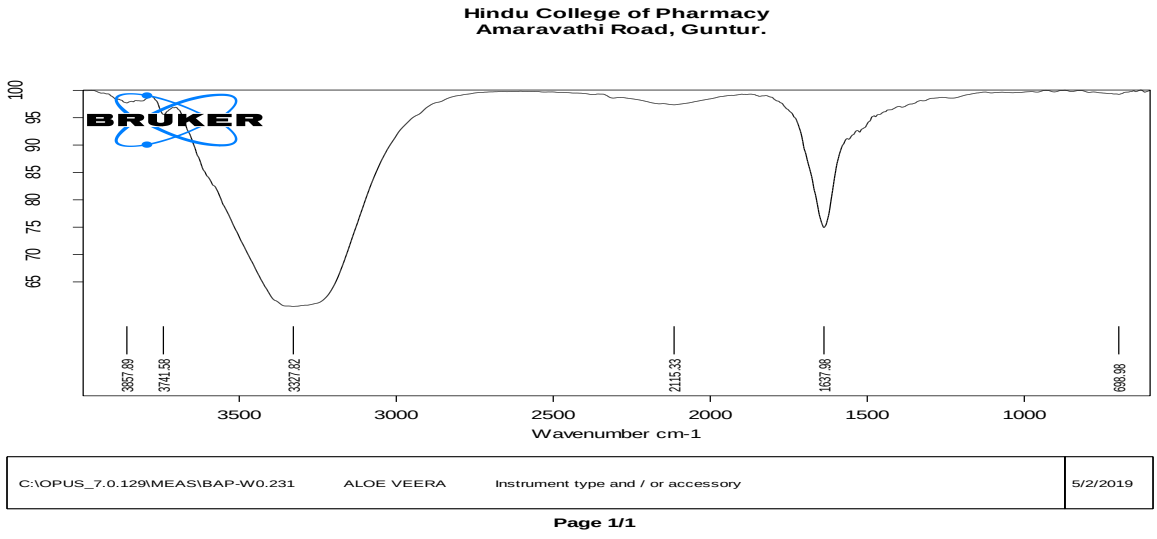


Fig 2 : FT-IR Spectrum of etanolic extract of Aloevera.

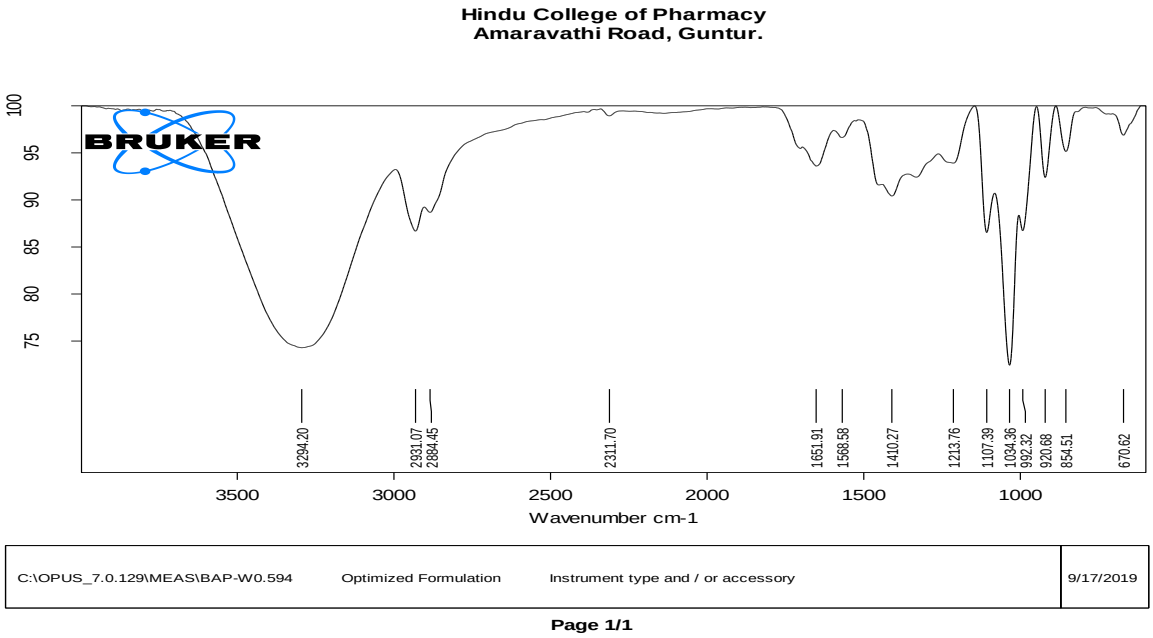


Fig 3: FT-IR Spectrum of optimized formulation.

Calibration curve of ethanolic extract of Eucalyptus globulus in phosphate buffer of pH 7.4

Standard plot of Eucalyptus globulus was plotted as per the procedure in experimental methods and its linearity was shown in tab 6.3 and fig 6.10 The standard graph of Eucalyptus globulus shows good linearity with R2 value of 0.997, which indicates that it obey's Beer's Lambert's Law in the concentration range of 0-100µg/ml.

Table 1: Calibration curve of Ethanolic extract of Eucalyptus globulus at 7.4.

Concentration (µg/ml)	Absorbance (276nm)
0	0
10	0.211
20	0.416
30	0.587
40	0.819
50	0.973

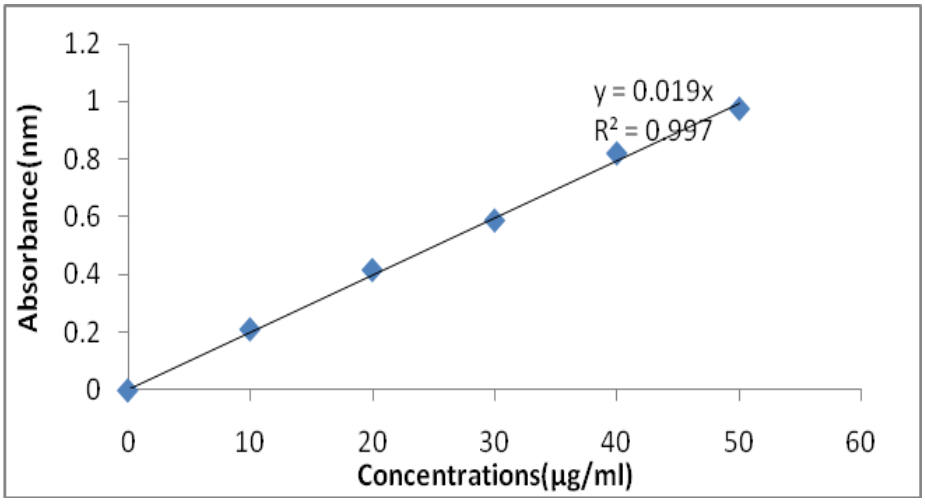


Fig 4: Calibration curve of ethanolic extract of Eucalyptus globulus in phosphate buffer of pH 7.4.

Calibration curve of ethanolic extract of Aloevera in phosphate buffer of pH 7.4:

Standard plot of Aloe barbidense was plotted as per the procedure in experimental methods and its linearity was shown in Table 6.5 and fig 6.12. The standard graph of Aloe bbarbidense shows good linearity with R2 value of 0.998, which indicates that it obeys Beer's Lambert's Law in the concentrate range of 0-100µg/ml.

Table 2 Calibration curve of ethanolic extract of Aloevera at pH 7.4.

Concentration (µg/ml)	Absorbance (220nm)
0	0
10	0.143
20	0.315
30	0.495
40	0.673
50	0.832

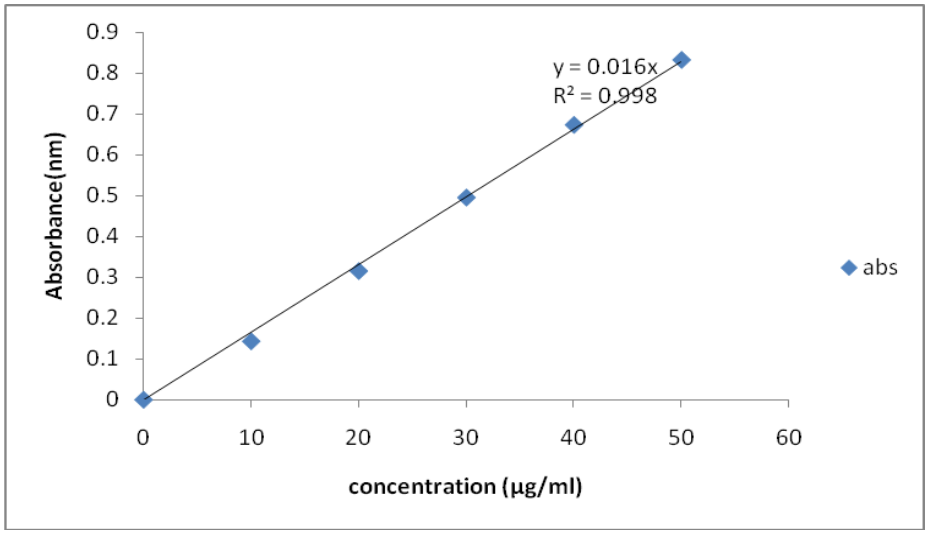


Fig 5: Calibration curve of ethanolic extract of Aloevera in phosphate buffer in 7.4.

Physical appearance:

All the formulations were evaluated for their colour and appearance. The physical appearance of all the formulations were found to be yellowish brown.

Extrudability studies:

Extrudability studies of all formulations were carried out as per procedure stated in methodology section. All the formulations having excellent extrudability.

Table 3: Characterization of cream formulations for colour, appearance and extrudability.

Formulation	Colour	Appearance	Extrudability
CF1	Brownish white	Semi-solid	Excellent
CF2	Brownish white	Semi-solid	Excellent
CF3	Brownish white	Semi-solid	Excellent
CF4	Brownish white	Semi-solid	Excellent
CF5	Brownish white	Semi-solid	Excellent
CF6	Brownish white	Semi-solid	Excellent
CF7	Brownish white	Semi-solid	Excellent

pH Determination :

Skin compatability is the primary requirements for a good topical formulation. It was found that the pH of all formulations were in the range of pH 5.4-7(Table 4) which indicates skin compatability i.e., creams can be applied to the skin without any discomfort or irritation.

Spreadability studies :

Spreadability study is one of the criteria for cream to meet the ideal qualities that it should posses good spreadability. If spreadability value is more, it would be properly spread over the skin which is more beneficial as per patient compliance concern. All the formulations were checked for the spreadability and the data was given in the table 6.9. The values of the spreadability indicated that the cream were easily spreadable by small amount of shear.

Rheological study:

The viscosities of all the formulations were measured using Brookfield viscometer, (spindle 42) (DV++) at 0.5 and 1 rpm and the viscosities for all the formulations were given in the table 4. It was found that all the formulations followed shear thinning effect with thixotropic property.

Drug content determination:

Drug content of all the formulations was carried out as per procedure stated in the methodology section. Drug content of all the formulations were found to be in the range of 99-100% as indicated in the table 4. The results indicates that uniform amount of drug is present in all the emulgel formulations.

Table 4 : Charecterization of cream formulations for pH, Spreadability, Viscosity and drug content.

Formulation	pH	Spreadability(cm /sec)	Viscosity(cps)		% Drug content (Eucalyptus)	% Drug content (Aloevera)
			0.5rpm	1 rpm		
CF1	5.8	19.08±0.11	195.1	183.3	97.92±0.12	97.81±0.1
CF2	6.1	18.03±0.32	192.7	189.1	99.22±0.5	99.06±0.6
CF3	5.6	19±0.56	190.4	176.8	100.12±0.3	100.56±0.01
CF4	6.0	17.04±0.12	193.3	200	98.75±0.8	-
CF5	6.2	18.59±0.25	194.0	192.4	-	99.64±0.2
CF6	5.7	19.05±0.54	192.5	210.5	98.51±0.09	-
CF7	6.6	18.09±0.85	192.9	206.6	-	98.0.07±0.07

***In –vitro* drug permeation for cream formulations:**

The cream formulations (EF1-EF7) were characterized for their drug diffusion study using Franz diffusion cell through a membrane and the drug content was calculated by simultaneous estimation method. The release studies of Eucalyptus and Aloevera from the prepared cream were performed in order to study the effect of different types and concentration on the release aiming to select the best formulation.

The release of the Eucalyptus from its cream formulations CF1,CF2,CF3,CF4 and CF6 can be ranked in the following descending order:CF3>CF1>CF2>CF4>CF6, where as the release of Aloevera from the cream formulation CF1,CF2,CF3,CF5 and CF7 can be ranked in the following descending order: CF3>CF1>CF2>CF7>CF5

These results suggested that CF3 was effective for topical application as highest percentage of drug released after 8hrs(table 5 and 6) in both the cases.

CF3 has shown high spreadability (19 cm/sec), less viscosity (190.4), excellent extrudability, more drug content(100%) and highest drug release than all other cream formulations. So, it was selected as the optimized formulation.

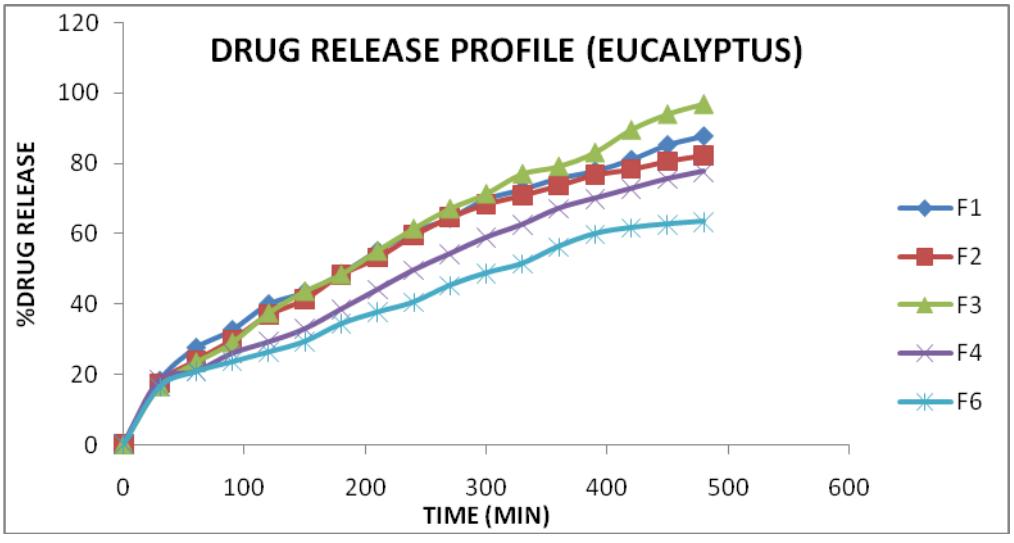


Fig 6 : Drug release profile of cream formulation containing Eucalyptus.

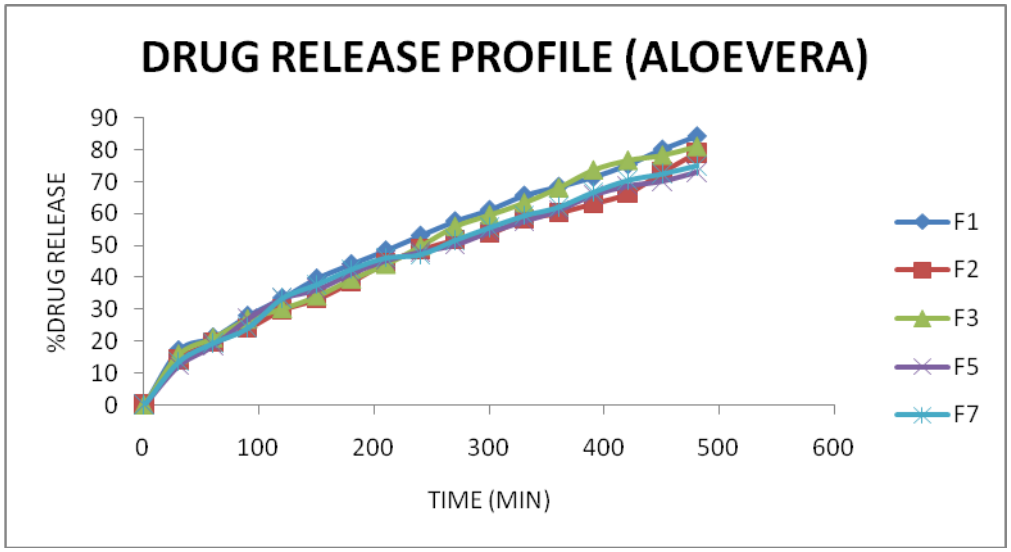


Fig 7 : Drug release profiles of cream formulations containing Aloevera.

Release mechanisms:

By incorporating release data in Higuchi and erosion models, the R2 values of all the formulations were found to be greater for Higuchi model. So, all the formulations in this study were best expressed by Higuchi's classical diffusion equation. The linearity of plot indicated that the release process was diffusion controlled.

To further comfirm the exact mechanism of drug release, the data was incorporated into koresmeyer peppas model and the mechanism of drug release was indicated according to the value of exponent 'n'. For all the cream formulations the release exponent 'n' value found to be between 0.696 and 0.739. This indicates the drug released from all the cream formulations followed non-fickian diffusion mechanism.

Table 5: Correlation coefficient values of cream formulations containing Eucalyptus.

Formulation	Zero order(R^2)	First order (R^2)	Higuchi (R^2)	Erosion (R^2)	Koresmeyer peppas	
					R^2	n
CF3	0.897	0.928	0.961	0.680	0.995	0.749

Table 6: Correlation coefficient values of cream formulation containing Aloevera.

Formulation	Zero order(R^2)	First order (R^2)	Higuchi (R^2)	Erosion (R^2)	Koresmeyer peppas	
					R^2	n
CF3	0.932	0.982	0.955	0.677	0.992	0.718

***In –Vivo* animal studies:**

Skin Irritation Test

The animal studies was started after getting IAEC permission ( ). The optimized formulation CF3 was applied on the hair free skin of the rats. The applied area is observed for 7 days and the results shown that the primary irritation index of the sample was 0.00 i.e., no erythema,edema, and no irritation were observed on the skin of the rabbit. The formulation is safe for topical use.

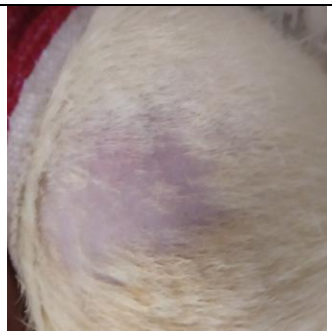
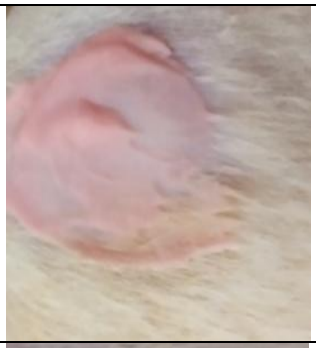
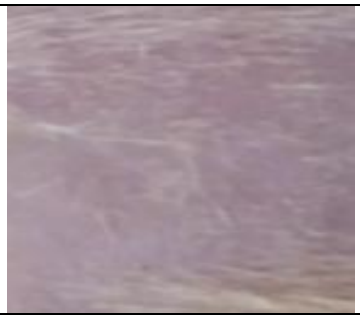
	Control	Standard	Test
Day 1			
Day 7			

Fig 8: skin irritation activity images.

Stability studies:

Stability study was performed on optimized batch CF3 at ambient conditions. The results obtained after 3 months time period were shown in the table. The results revealed that there is no significant change in pH, colour and drug content for optimized formulation. The results conclude that the optimized formulation is stable for 3 months and can be applied topically.

Table 7 : Short term stability studies of optimized formulation.

Formulation	Temperature	Initial		After 3months	
		pH	Drugcontent %	pH	Drug content %
Optimized formulation	4-8ºC	6.52	100±0.01	6.52	100±0.01
	25±2ºC	6.52	100±0.03	6.52	99±0.12
	45±2ºC	6.52	100±0.04	6.52	99±0.21

CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. In this study the topical application of the optimized formulation having anti bacterial activity of the extracts of Eucalyptus globulus and Aloevera. The antibacterial activity was well maintained when it was converted into cream formulation. The optimized formulation was evaluated on the basis of anti-acne property and skin irritancy property. The results shows that the above formulation was very effective for the treatment of acne vulgaris.

REFFERENCES

1. Leyden JJ: Therapy for Acne vulgaris. The New Eng J Med. 1997; 156-1162.
2. Collier CN, Harper JC, Cafardi JA, Cantrell WC, Wang W, Foster KW, et al. The prevalence of acne in adults 20 years and older. J Am Acad Dermatol 2008;58(1):56-9.
3. Akash S. Mali, Karekar. P, Dr. Yadav. A. V, Formulation and evaluation of multipurpose herbal cream, International Journal of Science and Research, 2013, 6(14).
4. Jagatheswari D, Deepa J, Sheik Jahabar Ali H *et al., Acalypha indica* L- An important medicinal plant; a review of its important traditional uses, and pharmacological properties. International Journal of Botany, 2013, 3(1) 19-122.
5. Sadhika C, Spandana D. A novel approach in drug delivery system using dendrimers. Pharma. Innovation J. 2019;8(5):166-74.
6. Mahendran Sekar and Fouzia Hanim Abdul Halim1, Formulation and evaluation of natural anti-acne cream containing syzygium samarangense fruits extract, Annual research & Review in Biology, 17(3):1-7,2017.
7. Ansila S, Dr. Deepu S, Dr. M. A. Kuriachan, Evaluation of Anti Acne Potential of Prepared Cream Containing Extract of Selected South Indian Medicinal Plant, International journal of pharmacy and pharmaceutical research, Vol.:10, Issue:2, September 2017.
8. Muhammad Athar Abbasi, Ayesha Kausar, Aziz-ur-Rehman, Hina Saleem, Sadia Muhammad Jahangir, Sabahat Zahra Siddiqui and Viqar Uddin Ahmad, Preparation of new formulations of anti-acne creams and their efficacy, African Journal of Pharmacy and Pharmacology, Vol. 4(6), June 2010.

9. Mohammed Haneefa K.P, Abid Shahima Hanan K, Guru Prasad Mohanta, Chandini Nayar, Formulation and Evaluation of Herbal Emulgel of Pothos Scandens Linn for Burn Wound Healing Activity. J. Pharm. Sci. 2014; Vol. 6(2):63-67.
10. Rathore RPS, Nema RK, Formulation and Evaluation of Topical Gels of Ketoprofen, Asian. J. Phaarm. Clinical. Res. 2008; 1: 12-16.
11. Sivanageswararao Mekala, Naresh Kumar M, Lipika Das Nishmitha Shetty, Arul Amuthan, Venkatarao Vulli, Evaluation of Wound Healing Activity of Ethanolic Extract of Lantana camara in Streptozotocin Induced Diabetic Rats, Int J Pharm Sci; 2014; Vol 6,Issue 1: 631-633.
12. Tadros TF., Future Development in Cosmetic Formulations, *Int J Cos Sci*; 1992; 14 (3): 93-111.
13. Angala Parameswari S , Kothapalli Bannoth Chandra Sekhar, Design And Evaluation Of Ciprofloxacin Floating Tablets Using Thermal Sintering Technique, International Journal of Pharmaceutical Sciences and Research, 2020,11(4),1879-1884.
14. Krishna KC, Kathirvel S. Fabrication of drug eluting medical device for treating stenosis by 3D printing and dip coating using aspirin as a model drug. Journal of Drug Delivery and Therapeutics. 2019 Dec 15;9(6-s):148-154.
15. Jyothsna C. Optimization and Characterization of Fenugreek Seed Polymer Nanoparticles Loaded with Diltiazem HCl Nanoparticles by Desolvation Method. Journal of Drug Delivery and Therapeutics. 2019 Dec 14;9(6-s):155-163.
16. Krishna KC. Pharmaceutical Co-Crystals: An Overview on Synthesis and Regulatory Aspects. Journal of Drug Delivery and Therapeutics. 2019 Jul 15;9(4):623-8.
17. Shahana VP. A Comprehensive Validation Method and Development of RP-HPLC for Simultaneous Estimation of Metoprolol, Telmisartan and Chlorthalidonein Bulk and its Formulation. Asian Journal of Research in Chemistry. 2018;11(6):827-34.
18. Parveen P, Usha P, Sowjanya C, Sravika SV, Ragala B, Gayatri M. An Overview of Immunology-Systemic. American Journal of Phytomedicine and Clinical Therapeutics. 2013; 644.

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