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Evaluation of Anti Acne Potential of Prepared Cream Containing Extract of Selected South Indian Medicinal Plant

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ABSTRACT

Herbal formulations have growing demand in the world market. The present work deals with the development and evaluation of the herbal Anti-Acne cream containing ethanolic extract of *Acalypha indica* and aloe vera gel. The plants have been reported in the literature having good antimicrobial, anti-oxidant and anti-inflammatory activity. Acne vulgaris, which is a skin disorder of the pilosebaceous gland leads to the formation of inflammatory lesions, seborrhea, comedone, etc. The pus being formed in acne which triggers inflammation is due to *Propionibacterium acne* and *Staphylococcus epidermidis*. Extract was prepared by Soxhlet extraction procedure and MIC was determined by broth dilution method. Seven batches of oil in water (O/W) herbal creams namely F1 to F7 were formulated by incorporating different polymers, stearic acid and cetyl alcohol. All the formulations were evaluated for various parameters like colour, appearance, consistency, irritancy, washability, pH, spreadability, drug content, drug release studies and antimicrobial activity. Rheological properties and kinetic properties of optimized formulation were studied. Among all the studied formulation, batch F4 was found optimum for all the parameters. It was a very good attempt to establish the herbal cream containing ethanolic extract of kuppaimeni leaves (*Acalypha indica*) and aloe vera gel.

INTRODUCTION

One of the most common disorders found among youngsters usually 18 - 25 years of age is Acne. Acne vulgaris, which is a skin disorder of the pilosebaceous gland leads to the formation of inflammatory lesions, seborrhea, comedone, etc. The resulting appearance can lead to anxiety, reduced self-esteem and in extreme cases depression or thoughts of suicide.[1]The pus being formed in acne which triggers inflammation is due to *Propionibacterium acnes* and *Staphylococcus epidermis*.Many treatment options are available to improve the appearance of acne including lifestyle changes procedures and medications. Eating fewer simple carbohydrates like sugar may help. Topical azelaic acid, benzoyl peroxide and salicylic acid are commonly used treatments. Antibiotics and retinoids are available in both topical and oral formulations to treat acne. However, resistance to antibiotics may develop. A number of birth control pills may be useful for preventing acne in women. Oral isotretinoin is usually reserved for severe acne due to greater potential side effects. Early and aggressive treatment is advocated by some to lessen the overall long-term impact to individuals.[2]

Indian herbs and its significance are popular worldwide. Herbal cosmetic's have growing demand in the world market and is an invaluable gift of nature. Herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser or nil side effects when compared to synthetic drugs. Nowadays the usefulness of herbs in the cosmeceutical production has been extensively increased in personal care system and there is a great demand for the herbal cosmetics in the market.[3]

In this study two south Indian herbs are used, they are *Acalypha indica* and *Aloe barbidensis*. Kuppaimeni leaves or Indian *Acalypha Indicia* (Biological term) is a wild plant having catkin type of inflorescence which carries various health saving medicinal properties.[4] The leaves of this plant are used in the treatment of many health problems especially respiratory diseases. Aloe vera is the colourless mucilaginous gel obtained from the parenchymatous cells in the fresh leaves of A. vera (L) burm. f. (Liliaceae) also known as *Aloe barbadensis*. Numerous scientific studies on A. vera demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, antibacterial and antifungal properties.

MATERIALS AND METHODS

The plant materials required for the study was collected from the region of Kazhakkuttam, Trivandrum and was authenticated by Department of Botany, University of Kerala, Kariyavattomcampus, Thiruvananthapuram.

- **Extraction of leaves of *Acalypha indica***

Plant extract of *Acalypha indica* was prepared using soxhlet extraction method. First the leaves were separated from plant washed with water and air-dried under shade. Dried leaves were pulverized using domestic grinder and the powder was used for extraction 250g of *Acalypha indica*leaves were first defatted using hexane and extracted with 1000 ml of methanol using Soxhlet apparatus. The extraction was carried out for 72 hrs at a rate of 3 cycles per hour and the extract was thereafter concentrated. The concentrated mass was kept at room temperature to remove methanol by evaporation and finally warmed at 45-50^{0}C to remove traces of methanol.[7]

- **Preparation of gel of aloe vera**

The fully expanded leaves of *Aloe vera* were selected from the plants and were washed with distilled water. The parenchymatous covering of the leaves were peeled and the gel drained out.

The obtained pulp was further crushed in a mechanical crusher. After crushing of the pulp it was filtered in order to remove the attached fibers. The obtained sap was collected and stored at 4^{0}C for further use.[6]

Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) values were determined by broth dilution assay. The cultures were prepared at 48 h broth cultures of *Propionibacterium acnes*. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. Seven sterile test tubes with 9 ml sterile nutrient broth were taken. 1ml of different concentration of drug solution was added and 0.1ml inoculum was also added to the test tube aseptically and media blank with the nutrient broth and the drug solution was also prepared. A positive control, containing media with 0.1ml inoculum was maintained to indicate the

growth promotion capacity of the media. Test sample was incubated under anaerobic condition in an anaerobic jar (Hi- Media) with gas pack for 48hrs.[8]

Formulation of cream by emulsification method[9]

Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (Cetyl alcohol, almond oil) were dissolved in the oil phase (Part A) and heated to 75ºC. The preservatives and other water soluble components (Polymer, Methylparaben, Triethanolamine, glycerol, ethanol extract of kuppaimeni and aloe vera gel) were dissolved in the aqueous phase (Part B) and heated to 75ºC. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring. An equivalent vigorous high shear type of mixing is equally effective. Stirring with the help of electrical stirrer was kept on until a homogenous cream was formed. After, the cream was allowed to cool to room temperature and then perfumes were added below 35ºC. The formula for the cream is given in table 1.

Table 1: Formula for the cream

Ingredients	**Formula % w/w**						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Ethanol extract of *A.indica*	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Ethanol extract of Aloe vera	0.5	0.5	-	0.5	-	0.5	-
Stearic acid	8	8	8	8	8	8	8
Cetyl alcohol	4	4	4	4	4	4	4
Poloxamer	-	0.1	0.1	-	-	-	-
Gellan gum	-	-	-	0.1	0.1	-	-
Methyl cellulose	-	-	-	-	-	0.1	0.1
Glycerol	3	3	3	3	3	3	3
Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tri ethanol amine	qs	qs	qs	qs	qs	qs	qs
Rosewater	qs	qs	qs	qs	qs	qs	qs

Evaluation of cream

- **Physical Properties:** The prepared cream was observed for color, odour, and appearance.

- **pH of the cream:** The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.[10]

- **Homogeneity:** Creams should be homogenous and pleasant in appearance. The formulations were tested for the homogeneity by visual appearance and by touch.[11]

- **Type of smear:** Prepared formulation was applied on the skin of the dorsal side of the hand. After that, the types of film or smear formed on the skin were checked.

- **Removal:** The ease of removal of the cream applied was examined by washing the applied part with tap water.[9]

- **Irritancy test:** Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.[9]

- **Spreadability test:** Two glass slides of standard dimensions were selected. The formulation whose spreadability had to be determined was placed over one of the slides. The other slide was placed on top of the formulations was sandwiched between the two slides across the length of 5 cm along the slide. 100g weight was placed upon the upper slide so that the formulation 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. One of the slides was fixed on which the formulation was placed. The second movable slide was placed over it, with one end tied to a string to which load could be applied with the help of a simple pulley and a pan. A 30g weight was put on the pan and the time taken for the upper slide to travel the distance of 5.0cm and separate away from the lower slide under the direction of the weight was noted. The spreadability was then calculated from the following formula: [12]

Spreadability= m ×l /t

m = weight tied to the upper slide (30g)

l =length of glass slide (5cm)

t =time taken in seconds

- **Test for microbial growth in formulation:**

The formulated creams were inoculated on the plates of agar media by streak plate method and a control was prepared by omitting the cream. The plates were placed into the incubator and are incubated at 37 ^{0}C for 24 hrs. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

Drug content: Each formulation (1gm) was accuratelyweighed and transferred to 100 ml volumetric flask towhich about 20 ml of acetone was added. Aftershaking, the volume was made up to 100 ml withpH 6.8 PBS. The content was filtered through a suitablefilter paper. 1ml filtrate was taken and suitably dilutedand the drug content (extract) was estimated by using UV/Visible spectrophotometerat 580nm. Results givenin the table are the average of triplicate values. Drugcontents values are expressed as Mean ± Standarddeviation.[8]

***In vitro* drug release studies:** *In vitro* release study of cream was carried out by using Franz diffusion cell. The formulation placed in donor compartment and 20:80 mixture of acetone and PBS pH 6.8 was taken in receptor compartment. Cellophane membrane, previously soaked overnight in the diffusion medium (PBS pH 6.8), was placed in between the donor and receptor compartment. 1g of the formulation was spread uniformly on a cellophane membrane, which was in contact with receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The receptor medium was stirred continuously with the magnetic stirrer. The temperature of the medium was maintained at 37±0.5 ^{0}C. at specific interval, 2 ml of sample was withdrawn from the receptor compartment and replaced with equal volume of PBS pH 6.8. Then analyzed by UV/Vis spectrophotometer at 580 nm.[8]

- **Rheological studies[16]**

Rheology means the study of the deformation and flow of matter. The deformation will depend on the properties of the material.Which property dominates, and what the values of the parameters are, depend on the stress and the duration of stress application Semisolid

formulations are best studied through rheological properties that relate stress and strain rate under different flow conditions such as oscillatory shear, rotational shear etc which are measured using rheometers.Dynamic oscillation frequency sweep test was used to determine thecapability of the cream to resist structural changes and the viscoelastic properties of the cream under the increased frequency.

The rheological properties of the prepared cream were determined using Rheometer MCR51 by frequency sweep analysis. About 0.5g of the formulation was applied to the plate and left for equilibrium. Measurements were made at fixed temperature 25^0C at the angular frequency (shear rate) ranging from 100-0.631 rad/s.

Using the data obtained from rheological studies, following plots were made :

1. Angular frequency Vs Storage modulus
2. Angular frequency Vs Loss modulus
3. Angular frequency Vs Complex viscosity

Antimicrobial activity:[14]

***Microorganisms*:** The microorganisms used in this study were bacteria (*propionibacterium acne*). Bacteria were cultured and maintained on Mueller-Hinton Agar (Merck, Germany) at 25^0C.

***Disk Diffusion Method*:** An antimicrobial assay was performed by using the Kirby-Bauer disc diffusion agar method. Agar plates were prepared by pouring freshly prepared agar medium to the sterilized Petri dishes after autoclaving. The microbial suspension was applied onto the solidified agars by using sterile cotton swabs and allowed to dry for 10 minutes. Formulated cream impregnated discs were aseptically transferred to the inoculated agar plates and left to be incubated for 2 days. The clear zones of inhibition around the test sample disc were measured for any indication of antimicrobial activity. Clindamycin impregnated discs were used as the standard reference or positive controls and the solvents were used as negative controls. All assays were carried out in triplicate.

Stability studies:[15]

Stability of a drug has been defined as the stability of a formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications throughout the shelf life. Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The cream filled in the bottle and kept in the humidity chamber maintained at 30 ± 2^0C/ 65 ± 5 % RH and 40 ± 2^0C / 75 ± 5 % RH for one month. At the end of studies, samples were analyzed for the physical properties, pH, drug content and drug release.

RESULT AND DISCUSSION

Determination of minimum inhibitory concentration: MIC was obtained from broth dilution method. MIC of ethanolic extract of kuppaimeni for *propionibacterium acne* was found to be 0.740 mg/ml.

Evaluation of cream

Physical properties of the cream

Figure 1. Prepared cream

Table 2: physical properties of cream

Formulation code	Colour	Odour	Appearance
F1	Yellowish white	Characteristic	Semi-solid
F2	Yellowish white	Characteristic	Semi-solid
F3	Yellowish white	Characteristic	Semi-solid
F4	Yellowish white	Characteristic	Semi-solid
F5	Yellowish white	Characteristic	Semi-solid
F6	Yellowish white	Characteristic	Semi-solid
F7	Yellowish white	Characteristic	Semi-solid

p^H of the cream: The pH of the formulations was found to be satisfactory and in the range of 5.8-6.5

Table 3: pH of cream

Formulation code	pH
F1	5.8
F2	5.9
F3	6.0
F4	5.6
F5	5.7
F6	6.2
F7	6.1

Homogeneity: All formulations produce the uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch.

Table 4: Homogeneity of cream

Formulation code	Homogeneity
F1	Good
F2	Good
F3	Good
F4	Good
F5	Good
F6	Good
F7	Good

Type of smear: Types of smear given by all formulations were tested, all give a non greasy film.

Table 5: Type of smear

Formulation code	Type of smear
F1	Non greasy
F2	Non greasy
F3	Non greasy
F4	Non greasy
F5	Non greasy
F6	Non greasy
F7	Non greasy

Removal: All formulations were found to be easy to wash with water.

Table 6: Removal of cream

Formulation code	Removal
F1	Easy
F2	Easy
F3	Easy
F4	Easy
F5	Easy
F6	Easy
F7	Easy

Irritancy test: All formulations were tested. None of the formulation shows redness, edema, inflammation, and irritation during irritancy studies. These formulations are safe to use for skin.

Table 7: Irritancy test of cream

Formulation code	Irritant	Edema	Erythema
F1	NIL	NIL	NIL
F2	NIL	NIL	NIL
F3	NIL	NIL	NIL
F4	NIL	NIL	NIL
F5	NIL	NIL	NIL
F6	NIL	NIL	NIL
F7	NIL	NIL	NIL

Spreadability test: The spreadability studies showed that all formulations have better spreadability when compared with the marketed cream. This is perfectly challenged to marketed Creams.

Table 8: Spreadability of cream

Formulation code	Time(sec)	Spreadability(g cm/sec)
F1	14	10.714
F2	15	10
F3	15	10
F4	**12**	**12.5**
F5	13	11.538
F6	13	11.538
F7	14	10.714
Marketed cream	13	11.538

All formulations have good spreadability. Among all F4 posses better value, F5 and F6 have spreadability comparable with marketed cream.

Test for microbial growth in formulation: The formulated creams were tested for the presence of pathogenic microorganisms by culturing it with agar medium using streak plate

method. There were no signs of microbial growth after an incubation period of 24 hours at 37^0C.

-^{VE} control After 24 hrs of incubation +^{VE} control

Figure 2: Test for microbial growth in formulation

Drug content: Drug content estimation of all formulations was carried out by using UV spectrophotometer at 580nm and was found to be in the range of 90 - 98%. The maximum % drug content was found to be 97.33 % in F1. The result of above studies are summarised in table 9

Table 9: Drug content estimation

Formulation code	Drug content(%)
F1	97.33±0.41
F2	96±0.52
F3	94.66±0.48
F4	96±0.22
F5	93.33±0.41
F6	92±0.28
F7	93.33±0.58

***In vitro* drug release studies:** The *in vitro* drug release studies were carried out using Franz diffusion cell for a period of 6 hrs. The percentage of drug released from the formulations F1-F4 were tabulated in table10 and F5-F7 were tabulated in table 11

Table 10: Percentage CDR data for formulations F1-F4 from receptor compartments

Time (hrs)	F1 % CDR	F2 %CDR	F3 %CDR	F4 %CDR
0	0	0	0	0
1	18.81±0.84	14.18±0.25	14.24±0.98	16.31±0.47
2	39.10±0.35	30.00±0.34	32.93±0.87	30.24±0.38
3	59.29±0.21	66.82±0.36	62.12±0.35	61.98±0.49
4	84.7±0.35	80.94±0.38	81.88±0.58	70.98±0.31
5	98.82±0.87	94.12±0.78	94.58±0.97	84.54±0.54
6	98.9±0.97	95.86±0.94	96.12±0.27	91.08±0.57

Table 11: Percentage CDR data for formulations F5-F7 from receptor compartments

Time (hrs)	F5 %CDR	F6 %CDR	F7 %CDR
0	0	0	0
1	15.65±0.24	15.78±0.47	14.90±0.57
2	28.14±0.67	30.69±0.38	34.21±0.28
3	54.27±0.98	54.38±0.25	56.13±0.38
4	70.15±0.64	71.05±0.67	73.68±0.67
5	82.15±0.32	89.48±0.37	90.34±0.47
6	92.17±0.12	94.14±0.17	93.12±0.15

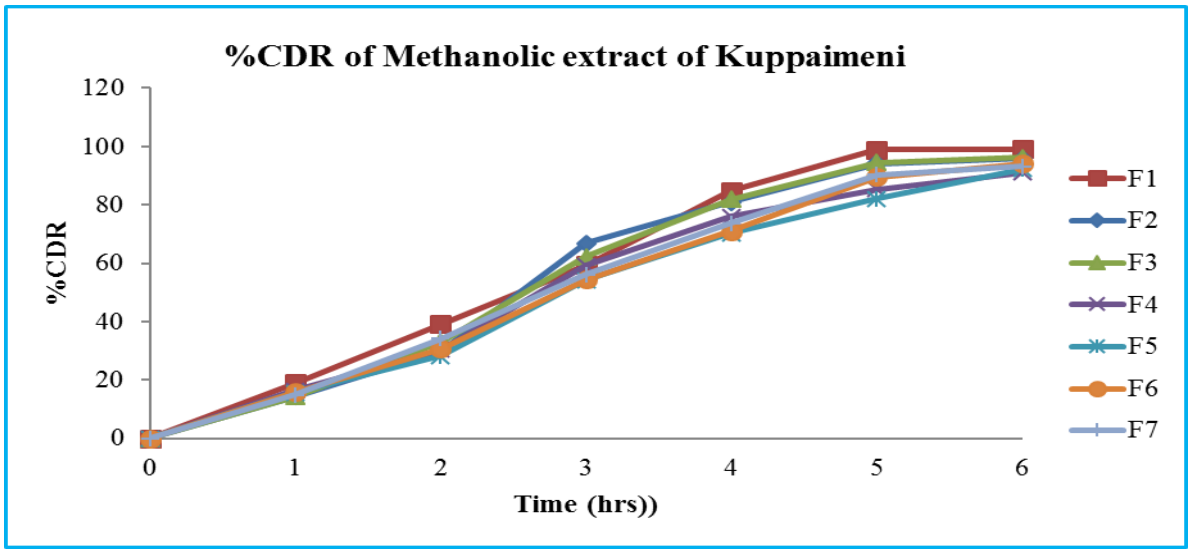


Figure 3: Percentage CDR profile of Kuppaimeniextract in formulations F1-F7

Formulations F1, F2, and F3 had a rapid onset of release and almost 80 % was released in 4 hours itself. Formulations F4 and F5 shows sustained release over 6 hours. Formulations F6 and F7 show slow release than F1 but rapid as compared with F4 and F5.

Rheological studies: Rheological properties of the cream F4 were studied by frequency sweep analysis. Following data are obtained.

Table 12: Rheological data of F4 obtained from frequency sweep analysis

Angular frequency (rad/s)	Storage Modulus (Pa)	Loss Modulus (Pa)	Complex Viscosity (Pa.s)	Log angular frequency (rad/s)	Log storage modulus (Pa)	Log loss modulus (Pa)	Log complex viscosity (Pa.s)
100	1790	1200	3.01	2	3.252853	3.079181	0.478566
63.1	903	830	4.57	1.800029	2.955688	2.919078	0.659916
39.8	499	522	6.25	1.599883	2.698101	2.717671	0.79588
25.1	226	275	8.15	1.399674	2.354108	2.439333	0.911158
15.8	93.3	124	11.4	1.198657	1.969882	2.093422	1.056905
10	33	61.2	17.8	1	1.518514	1.786751	1.25042
6.31	15.6	36.2	28.3	0.800029	1.193125	1.558709	1.451786
3.98	10.9	28.8	43.17	0.599883	1.037426	1.459392	1.635182
2.51	9.13	27	61.47	0.399674	0.960471	1.431364	1.788663
1.58	9.58	26.5	80.45	0.198657	0.981366	1.423246	1.905526
1	9.44	26.6	108.45	0	0.974972	1.424882	2.03523
0.631	9.05	27.4	137.04	-0.19997	0.956649	1.437751	2.136847

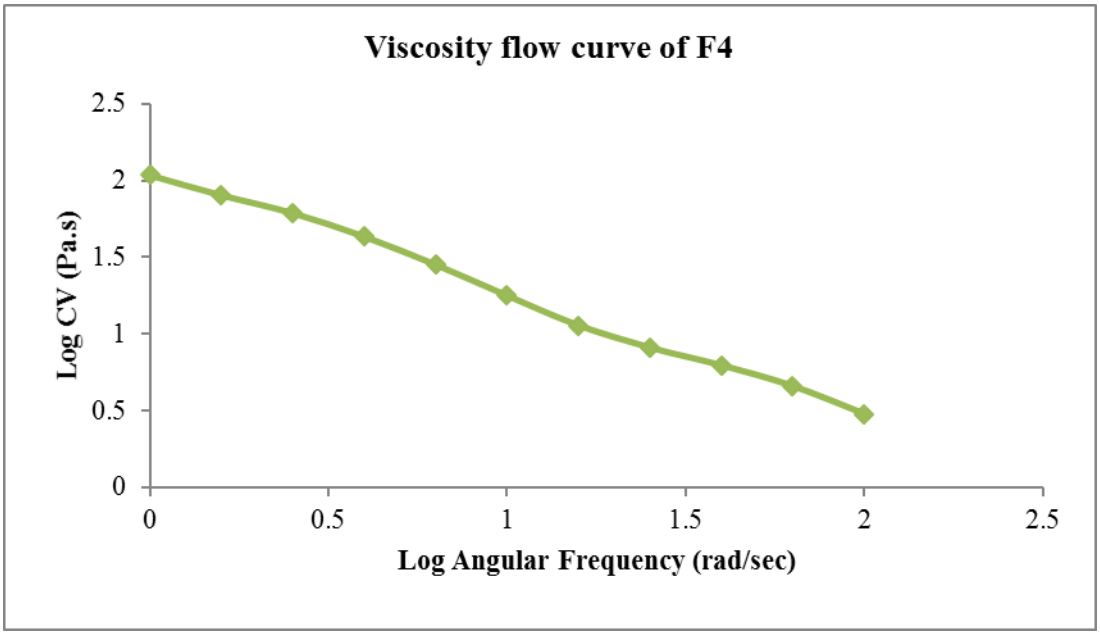


Figure 4: Viscosity flow curve of optimized formulation F4

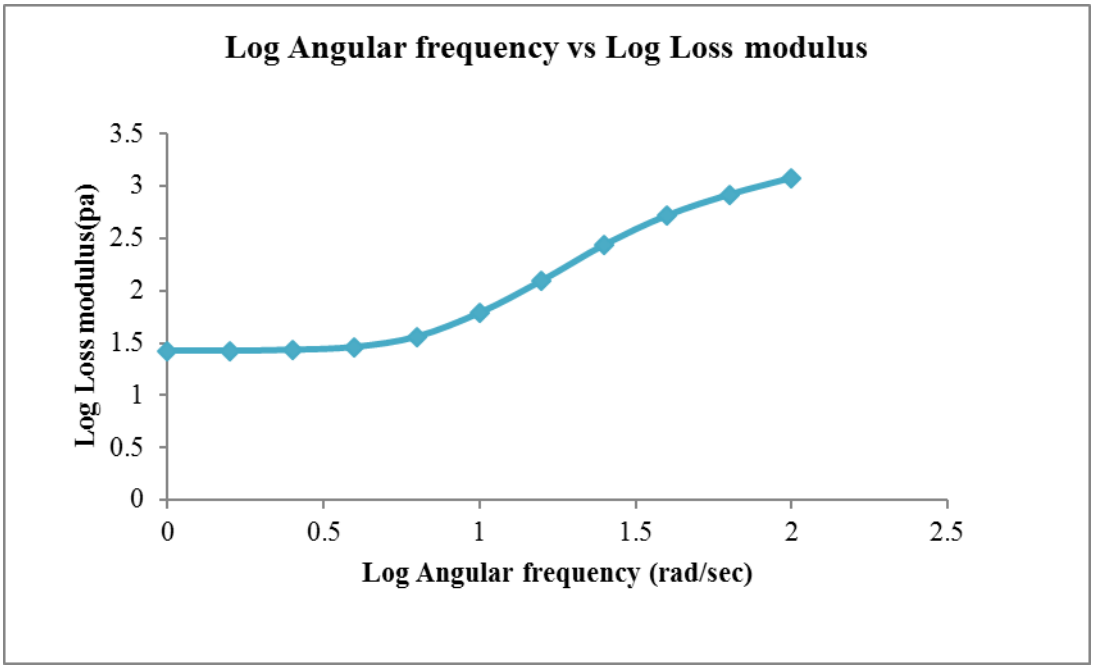


Figure 5: Graphical representation of Log Angular frequency Vs Log Loss modulus

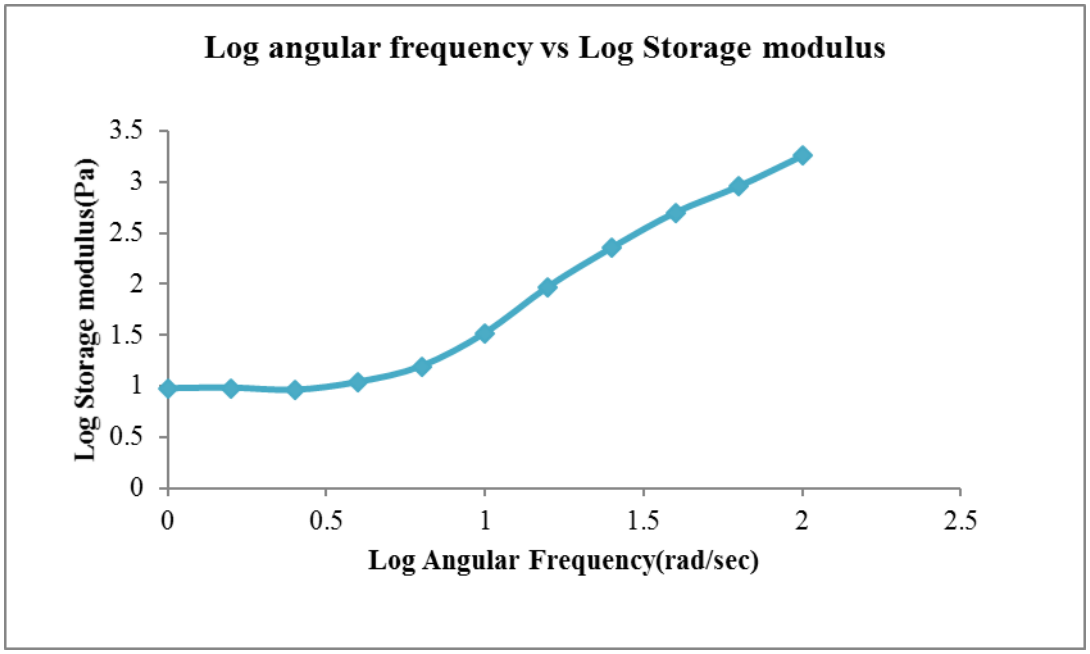


Figure 6: Graphical representation of Log Angular frequency Vs Log Storage Modulus

From the data, it was obtained that cream has the viscosity of 137.04 Pa.s at 0.631rad/sec shear rate, and 3.01 Pa.s at 100rad/sec shear rate.

From the viscosity flow curve of optimized formulation F4 shown in Figure 4, it was found that the formulation follows Newtonian flow indicating the decrease in complex viscosity at increasing angular frequency (shear rate). This decreased viscosity of the formulation, ie, the decline in resistance to flow due to the increasing shear rates indicated that the formulation shows shear-thinning behavior or pseudo-plasticity.

From the Figure 5, it was found that the loss modulus increased with increasing angular frequency. Loss modulus is the measure of energy dissipated due to the viscous flow.

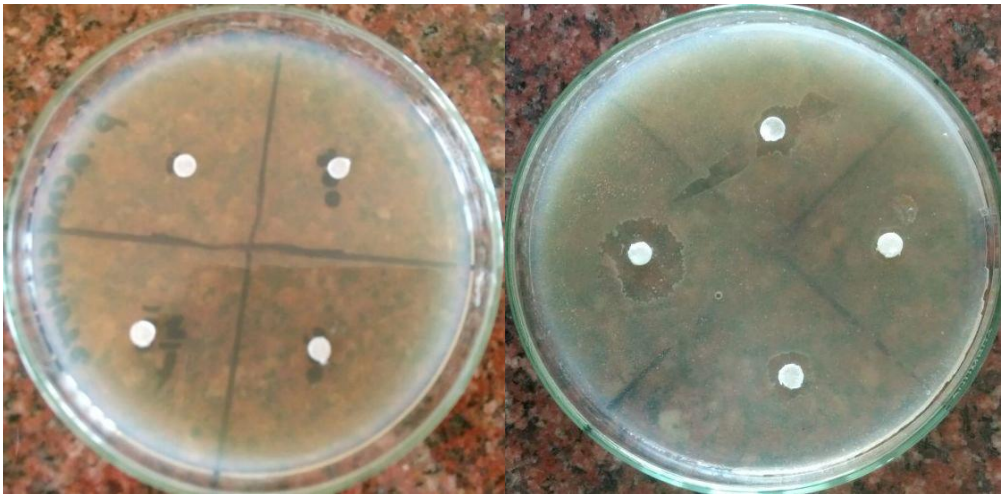
Figure 6 indicated that when the angular frequency was increased, the storage modulus also increased. Storage modulus is an indication of the gel's ability to store deformation energy in an elastic manner. This is directly related to the extent of cross-linking. Higher the degree of cross-linking, the greater storage modulus. Higher storage modulus means higher strength or mechanical rigidity.

Antimicrobial activity: Antimicrobial activity was carried out using Kirby-Bauer disc diffusion agar method. Test organism used for this test was *Propionibacterium acne*. The

table gives the zone of inhibition given by all formulations. It indicates, the extent of antimicrobial activity of formulations.

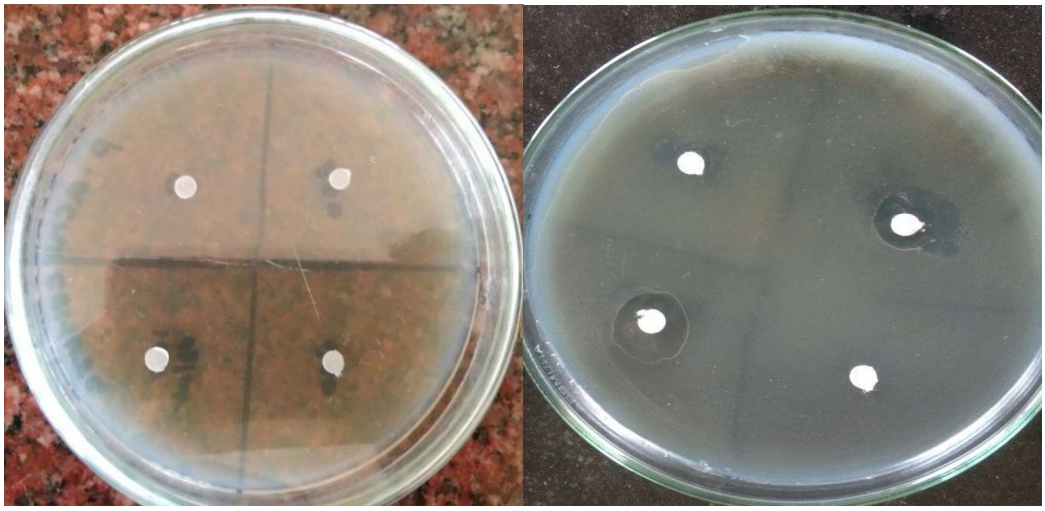
Table 13: Zone of inhibition produced by formulations F1-F7

Formulation code	Diameter of zone of inhibition (cm)	* - Extend of antimicrobial activity
F1	1	**
F2	1.1	**
F3	0.9	*
F4	1.3	***
F5	0.8	*
F6	1.2	**
F7	0.9	*
Clindamycin (10µg/ml)	1.4	***



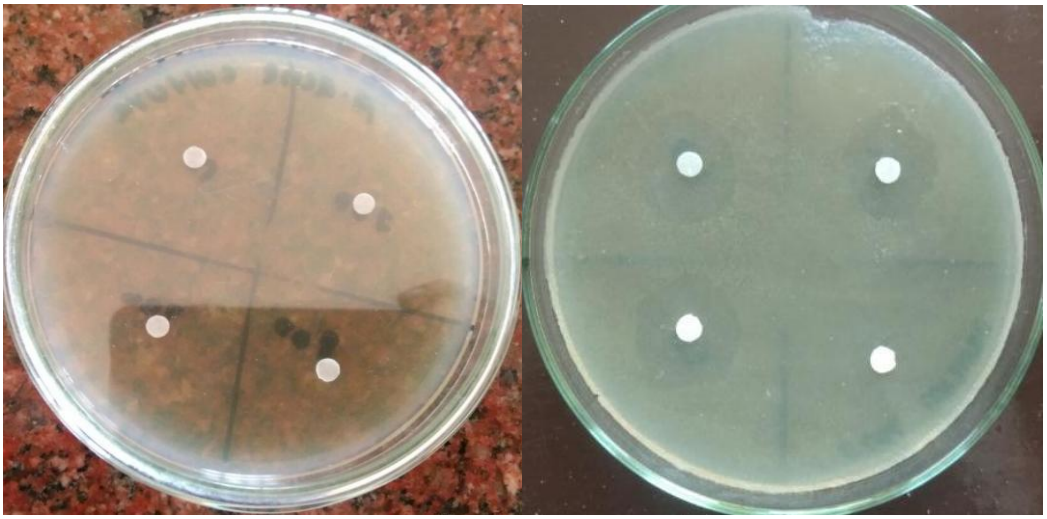
1st day After incubation

Figure 7 Inhibition zones produced by F2, F3 & F5



1st day After incubation

Figure 8 Inhibition zones produced by F1 & F7



1st day After incubation

Figure 9 Inhibition zones produced by F4, F6 & clindamycin

The developed formulations were evaluated for their *in vitro* antibacterial activity against *P. acnes*. The Zones of inhibitions produced by all formulations were compared with the standard clindamycin. Formulation F4 has shown comparable zones of inhibitions to that of clindamycin solution. Formulations F3, F5, and F7 were shown less zone of inhibition. That is formulations without aloe vera show less antimicrobial activity than the others. And also this result confirmed the anti-acne potential of formulated cream of kuppaimeni.

5.7.14 Stability studies

Stability studies were carried out on formulations F4 for a period of 1 month and comparison of the parameters before and after stability studies was reported in the table.

Table 14: Comparison of physical parameters before and after stability

Stability Period	Colour	Appearance	Homogeneity
Before stability study	Yellowish white	Semi-solid	Good
After stability study	yellow	Semi-solid	Good

Table 15: Comparison of pH and drug content before and after stability studies

Stability Period	pH	Drug content
Before stability study	5.8	96
After stability study	5.5	95.14

Table 16: Drug release determination after stability

Time (hrs)	% CDR	
	Before stability study	After stability study
0	0	0
1	16.31	14.5
2	30.24	28.75
3	61.98	57.24
4	75.98	73.48
5	84.54	82.29
6	91.08	90.75

From the stability studies, it was found that there was a slight color change in the formulation and stable in case of appearance and odour. Also, there was slight decrease in the pH, drug content, and drug release after 1 month. But it was not a large difference to affect the activity of formulation. So it could be concluded that the prepared cream have adequate stability.

CONCLUSION

In the present study, an attempt was made to formulate herbal cream using different polymers by incorporating the ethanolic extract of *Acalypha indica* and aloe vera gel and to evaluate the prepared cream for the desired parameters. Seven different formulations of three different polymers were prepared and evaluated for physical parameters, rheological studies, drug release studies, antimicrobial study and stability studies. All of the formulations possess better physical properties, consistency, washability, pH and they are safe to apply on skin. Among these formulations containing polymers have the ability to sustain the release of the drug than the formulation without a polymer. Even though, F4 gives more sustained release. And also F4 had spreadability and rheological properties comparable to marketed creams. Also in the case of antimicrobial studies formulation F4 gives zone of inhibition comparable to clindamycin.Based on the results we conclude our work that the herbal cream prepared using gellan gum was found with acceptable properties than the others. From this study, it was confirmed the anti-acne potential of the kuppaimeni and synergistic action of aloe on it. The present research work can be suggested as an effective tool for treating Acne.

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