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Anti Acne Hydrogel of Coriandrum Sativum

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ABSTRACT

Acne is a disease appearing simple but chronic in nature with multiple causative factors like bacteria, inflammation, hormones etc. The present study was conducted to formulate and evaluate the topical anti acne formulation of coriander aqueous extract. The antibacterial activity of aqueous extract against *Propionibacterium acne* (*P. acne*) and *Staphylococcus epidermidis*(*S. epidermidis*) was investigated using disc diffusion method and minimum inhibitory concentration was determined by agar dilution method. The results showed that coriander aqueous extract showed the MIC values of 1.7 mg/ml and 2.1 mg/ml against *P.acne* and *S. epidermidis* respectively. The zone of inhibition exhibited by the aqueous extract was 21.5±1.4mm and 20.6±1.09mm against *P.acne* and *S. epidermidis* respectively. The topical formulations were developed using menthol as the penetration enhancer and tested for physical parameters, drug content uniformity, spreadibility, extrudability and *in-vitro* diffusion. It was reveled from the results that all the formulations showed the increased zone of inhibition for both of the bacteria. The formulations with the addition of penetration enhancer showed the increased drug content as well as the *invitro* release which increased with increase in the concentration of menthol. The formulation Fa_{11} showed the drug content (97.9%), *in-vitro*- diffusion (96.8%) and maximum stability among all the formulations. The optimized formulation showed the thixotropic behavior and first order release rate.

Keywords: acne vulgaris, antibacterial activity, coriander, penetration enhancer, *in-vitro* activity.

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INTRODUCTION

Acne is an inflammatory disease of sebaceous follicles of skin, marked by comedones, papules, and pustules [15] and presence of bacterias *Propionibacterium acne*, *Staphylococcus epidermidis* and *Malassezia furfur* in follicular canal [1] .*P. acne* is obligatory anaerobic organism residing in human skin as cutaneous flora. The oxidative stress within the pilosebaceous unit changes the environment from aerobic to anaerobic which is the best suited for this gram positive bacterium. [4] It is implicated in development of inflammatory acne as it activates complement and metabolize sebaceous triglycerides into fatty acids which chemo tactically attract neutrophills. *S. epidermidis* is aerobic organism involved in superficial infection within sebaceous unit.[1] Thus *P.acne* and *S.epidermidis* are target sites for anti acne drugs.

The treatment of acne is dependent on the kind of lesions as both oral as well as topical treatments are available. The mild and moderate acne are treated by topical therapy while severe acne is treated through oral antibiotics. The antibiotics are the mainstay for the treatment of acne[12, 30].

Gram-negative folliculitis can occur as a complication of any long term topical or oral antibacterial treatment resulting in sudden onset of multiple pustules frequently localized around the perioral and perinasal areas. The minimum duration of treatment for acne either by using topical or oral anti acne agents is two –three months [20, 18].

This long term expensive treatment increases the instances of alarming adverse effects. The increasing instances of bacterial resistances [23] further developed the surge to adopt an alternative treatment. Among the alternative treatments, natural therapy is the most acceptable. The natural therapy lacking adverse effects is highly desirable with respect to its conceivable safety and rare *P. acne* resistance. Naturally derived compounds, particularly those from herbs have been a good prospect for future development of anti acne products[3]. Among alternative treatment herbals are the best options with no side effects and are economic to use.

In the realm of allopathic, herbals have their own bench mark position. As the figures from the WHO suggest that 4 billion people, who make nearly 70 % of world population are the users of herbal medicines for some purpose of primary healthcare. This figure is continuously improving due to intense gray side of allopathic (side effects, less economic, bacterial resistance etc). The popularity of herbal medicines is growing by leaps and bounds in the global market. The global herbal market is of size 62 billion dollars [5,6]. So the development of herbal anti acne formulation is an advantageous step.

Menthol is a well established penetration enhancer reported to be non irritational to the skin and produce the cooling effect due to its negative heat of sublimation[10]. This property of it will give good feeling to the patient with inflammation in acne.

Coriandrum sativum is medicinally proved to have therapeutic activities like hypoglycemic, [21,28] anti-inflammatory, [7,8] Hypolipidemic, [17,26] antioxidant, [16] anti scarring property due to the presence of salicylic acid [2] and anti microbial activity against bacteria and fungi [22,27]. All these properties can contribute to an effective anti acne agent so present study is carried to investigate the anti acne property of coriander and to formulate and evaluate its topical anti acne formulation.

MATERIAL AND METHODS

The coriander leaves and dried seeds were purchased from the local markets of Modinagar. The plant material was authenticated by taxonomist at Modinagar and the specimens were deposited at Botanical section of M. M. PG College, Modinagar. The test organisms, *P.acne* (MTCC 1951) and *S. epidermidis* (MTCC 931), were obtained from Microbial culture collection and Gene bank, Chandigarh, India. All media were purchased from Hi-Media. All reagents used were of analytical grade.

Determination of antimicrobial activity:

Determination of antibacterial activity

The antibacterial activity was determined by disc diffusion method. This experiment was performed by following the method of Hayes and Markovic (2002) with some modifications. *Propionibacterium acne* was incubated in ASLA agar medium for 48 hrs under anaerobic conditions and adjusted to yield approximately $1x10^8$ CFU/ml. The agar plates were swabbed by with inoculums. 0.05% polysorbate 80 was added to the agar base used coriander oil. The sterile filter paper disc of diameter 6mm were aseptically placed on the inoculated plates and were impregnated with the test material (100mg/ml of extracts and 20µl of coriander oil). The plates were left at ambient temperature for 30 min to allow exceed pre diffusion prior to incubation at 37 ^{0}C for 72 hrs under anaerobic conditions in a anaerobic bag (Hi-Media) with gas pack and indicator tablets and the bag was kept in an incubator for 72 hrs at 37 + 1 ^{0}C . Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition. The culture of Staphylococcus epidermidis was prepared in nutrient agar medium at 24 hrs under aerobic

conditions. Test samples of this aerobic bacterium were incubated at 37^0C for 24 hrs under aerobic conditions. The anti bacterial activity was estimated by measuring the diameter of the zone of inhibition. All disc diffusion tests were performed in three separate experiments and antibacterial activity was expressed as the mean ± standard deviation [13,14,15].

Determination of Minimum inhibitory Concentration

The minimum inhibitory concentration (MIC) values were determined by agar dilution method. The test materials were added aseptically to 20ml aliquots of sterile molten agar (containing 0.05% polysorbate 80 in case of coriander oil)at appropriate range of test material(0.05mg/ml-5mg/ml for extract and 0.05- 3% v/v for coriander oil). The resulting agar solutions were vortexed at high speed for 15 secs or until completely dispersed, immediately poured into sterile petri plates then allowed to set for 30 min. The plates were then inoculated with the Propionibacterium acne. The inoculated plates were left until the inoculums had set and then incubated under anaerobic conditions at 37^0C for 72 hrs in gas bag (Hi-Media) with gas pack and indicator tablets and the bag was kept in an incubator for specified duration at specified temperature. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition. The test samples of Staphylococcus epidermidis were prepared in nutrient agar medium and incubated for 24 hrs at 37^0C under aerobic conditions. Following the incubation period, the plates were observed and recorded for the presence or absence of growth. From the results, the MIC was recorded as the lowest concentration of test substance where the absence of growth was observed [13,14,15].

Minimum bactericidal concentration (MBC) was determined by sub culturing the samples on to the sterile agar plates from the three test plates, with each of bacterium, which had shown no growth during the determination of MIC. The plates for each of bacterium were incubated following the same procedure as described in MIC determination. The minimum bactericidal concentration values were interpreted as the highest dilution (lowest concentration) of sample which showed no growth on agar plates [15,24,25].

Preparation of Calibration Curve

For the calibration curve of coriander aqueous extract, the stock solution was prepared in phosphate buffer pH 6.8. The specified amount of powdered aq. extract was dissolved in phosphate buffer pH 6.8 to obtain the stock solution of 1mg/ml. Later, the working samples

were prepared from this stock solution. The absorbance was taken at 239 nm using double beam UV spectrophotometer [36].

Partition Coefficient Determination

The partition coefficient of the drug (coriander aqueous extract) between octanol and water was determined at 25±1 ^{0} C. An excess amount of drug was taken in a separating funnel containing 1:1 of octanol and water and placed in a water bath for 24 hrs. The solution was shaken at regular intervals. Then, both of them were separated and filtered through a 2µg filter and the drug concentration in each phase was determined by measuring the absorbance using spectrophotometer at 239 nm.

Preparation of gel with penetration enhancer:

The weighed amount of methyl paraben was dissolved in 5ml of hot water and propyl paraben was added on slight cooling of water. To this beaker carbopol 934 was dispersed with continuous stirring for 20 min after addition of 50 ml of distilled water. This dispersion was kept overnight for soaking. In another beaker the required quantity of propylene glycol and polyethylene glycol [PEG 400] were added. To this mixture the drug (aqueous extract) corresponding to its MIC was also incorporated and finally this mixture along with menthol dissolved in ethanol, was added to the carbopol beaker with stirring. The volume was made up with distilled water and stirring was done vigorously. Triethanolamine was added form the gel by adjusting pH to 6.8 [10] . The same procedure was adopted for formulating the formulation without penetration enhancer but menthol and ethanol were not added.

Antimicrobial studies of the formulation:

The solutions of the gels were prepared using 100mg of gel in 10ml of dimethyl sulfoxide. The anti bacterial activity was tested by well diffusion method. *Propionibacterium acne* was incubated in ASLA agar medium for 48 hrs under anaerobic conditions and adjusted to yield approximately 1x10^8 CFU/ml. The solidified agar plates were swabbed with inoculums on the surface. The equidistance wells were cut in the plates with help of 8mm borer. In each of these wells the gel solutions were placed and the plates were left at ambient temperature for 30 min to allow pre diffusion prior to incubation at 37 ^{0}C for 72 hrs under anaerobic conditions in a anaerobic bag (Hi-Media) with gas pack and indicator tablets and the bag was kept in an incubator for 72 hrs at 37 ± 1 ^{0}C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition. The culture of *Staphylococcus epidermidis* was prepared in nutrient agar

medium at 24 hrs under aerobic conditions. Test samples of this aerobic bacterium were incubated at 37^0C for 24 hrs under aerobic conditions. The anti bacterial activity was estimated by measuring the diameter of the zone of inhibition. All well diffusion tests were performed in three separate experiments and antibacterial activity was expressed as the mean ± standard deviation [1].

Physical appearance:

The physical appearance of the formulation was checked visually which comprised of-:

Colour- The colour of the formulations was checked out against white background.

Consistency- The consistency was checked by applying on skin.

Greasiness- The greasiness was assessed by the application on to the skin.

Odour- The odour of the gels was checked by mixing the gel in water and taking the smell.

pH:

About 20mg of the formulation was taken in a beaker and was subjected to the pH measurement using a digital pH meter within 24 hrs of manufacture [31].

Viscosity:

Viscosities of formulated gels were determined using Brookfield viscometer spindle # 7 at 50 RPM and 25^0C. The corresponding dial reading on the viscometer was noted. Then the spindle was lowered successively. The dial reading was multiplied by the factor mentioned in catalog[31].

Extrudability:

Extrudability is defined as the weight in grams required for extruding 0.5cm long ribbon of formulation in 10 secs. The gel formulation was filled in a standard capped collapsible aluminum tubes and sealed by crimping to the end. The tubes were placed between two slides and were clamped. 500g weight was placed over the slides and then the cap was removed. The length of the ribbon of the formulation that came out in 10 secs was recorded [9].

Spreadibility:

Spreadibility denotes the extent of area to which a gel readily spreads on the application to the akin or affected part. The bioavailability efficiency of the gel also depends on Spreadibility value. Spreadibility is defined in terms of time in secs required taken by the upper slide to slip off the gel placed between the two slides, under certain load. The lesser the time taken for the separation of two slides, the better the spreadibility. About 500mg of the formulation was sand witched between the two slides, each with dimensions of 6x2cm. A weight of 100g was placed upon the upper slide so that the formulation between the two slides get pressured uniformly to form a thin layer. The weight was removed and the excess of the formulation adhering to the

slides was scrapped off. The lower slide was fixed on the board of apparatus and the upper slide was to the non-flexible string to which 20g load was applied with the help of a simple pulley which was in horizontal level with the fixed slide. The time taken by the upper slide to slip off the lower slide was noted [11].

$$\text{Spreadibility} = m \times l/t$$

Where, m= weight tied to upper slide, l= length of the glass slide (6cm), t= time in secs.

Syneresis

Syneresis is one of the major problems associated with gels. Syneresis means contraction of gel upon standing and separation of water from the gel. Syneresis is more pronounced in the gels where lower concentration of gelling agent is used. Gels were kept under scrutiny for signs of Syneresis. The gels showing signs of Syneresis were rejected and not considered for further studies. The degree of syneresis was observed at room temperature and in refrigerator also (2-8°C). Syneresis was observed after 24 hrs of gel preparation[5,7].

Rheological behavior

The rheological property was determined to know the flow behavior of formulation. The viscosity at different rmps was measured using Brookfield viscometer. The rheological behavior of formulation was studied by taking 100g gel in a beaker. The rate of shear was increased gradually from minimum to maximum and corresponding dial reading was noted. Then the rate of shear was decreased gradually to the lowest value and dial reading was noted. The graph was plotted between the torque and viscosity to determine type of flow[30].

Drug Content:

The drug content of the gel formulations was determined by dissolving an accurately weighed quantity 1gm of gel in 100ml of solvent (phosphate buffer pH 6.8 for formulations of aqueous extract of coriander and a mixture of ethanol and phosphate buffer pH 6.8 (60:40) for formulations of coriander oil). The solutions were kept for shaking for 4hrs and then kept for 6hrs for complete dissolution of the formulations. Then the solutions were filtered through 0.45mm membrane filters and proper dilutions were made and solutions were subjected to the Spectrophotometric analysis. The drug content was calculated from the linear regression equation obtained from the calibration data.

***In-vitro* diffusion studies**

The *in-vitro* diffusion studies for all formulations were carried out using the Franz diffusion cell with an area of 3.7994 cm^2 and 100m height, having a diffusion area of 3.8 cm^2. A weighed quantity of formulation equivalent to 1gm of the drug was placed onto the dialysis membrane-70

(Hi-Media) and was immersed slightly in 100ml of receptor medium (phosphate buffer pH6.8 for the formulations of coriander aqueous extract and mixture of ethanol: phosphate buffer pH 6.8 (60:40) for the formulations of coriander oil) which was continuously stirred and the temperature was maintained at 37^0C ±1^0C. Aliquots of 1ml were withdrawn from each of the system at time intervals of 5, 10, 15, 30, 60,120, 240,360 min and analyzed for drug content using UV spectrophotometer [9, 29].

Skin permeation studies

In-vitro skin permeation studies were carried out for the best four formulations that showed higher drug release through dialysis membrane-70, using the rat abdominal skin. The rat skin was obtained from the abdominal portion of an albino rat after sacrificing the animal. The hair and the fat were removed after treating the akin with 0.32 mol /l ammonia solution for 30 min. The excised rat skin was washed with receptor medium, tied to the Franz diffusion cell (donor cell) having a diffusion area of 3.8 cm^2 and 100cm height, so that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell. All experiments were carried out in triplicate. A weighed quantity of formulation equivalent to 1g of the drug was placed onto the rat skin and was immersed slightly in 100ml of receptor medium (phosphate buffer pH 6.8 for the formulations of coriander aqueous extract and mixture of ethanol: phosphate buffer pH 6.8 (60:40) for the formulations of coriander oil) which was continuously stirred and the temperature was maintained at 37^0C ±1^0C. Aliquots of 1ml were withdrawn from each of the system at time intervals of 5, 10, 15, 30, 60,120, 240,360 min and analyzed for drug content using UV spectrophotometer [35].

Stability studies:

The tubes of gels were kept at temperature of 2, 25, 40 ^{0} C for the specified period of 30 days, 60 days and 90 days as per ICH guidelines. Then these formulations were analysed for ph, viscosity, drug content, % cumulative drug release and zone of inhibition[25, 32].

Drug release kinetics

To study the release kinetics of the formulations, the data obtained from *invitro* release studies were plotted in various kinetic models.

Zero order as cumulative amount of drug released vs. time (equation 1)

$$C=K_0 t \quad (1)$$

Where, K_0 is the zero order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs. time with a slope equal to K_0 and intercept the origin of axes.

First order as log cumulative percent drug remaining vs. time (equation 2)

$$\text{Log } C = \text{Log } C_0 - k\,t/2.303 \qquad .(2)$$

Where, C_0 is the initial concentration of the drug, k is the first order rate constant and t is the time in hours.

Higuchi's model as the cumulative percentage of drug released vs. square root of the time (equation 3).

$$Q = K.\sqrt{t} \qquad (3)$$

Where, K is the constant reflecting the design variables of the system and t is the time in hours. Hence drug release rate is reciprocal of square root of time [34,35].

RESULT AND DISCUSSION

Antimicrobial activity of extract

The antibacterial activity of Coriander aqueous extract against *Propionibacterium acne* and *Staphylococcus epidermidis* was investigated using disc diffusion method and minimum inhibitory concentration was determined by agar dilution method. The results showed that coriander aqueous extract showed the MIC values of 1.7 mg/ml and 2.1 mg/ml against *P. acne* and *S. epidermidis* respectively. The zone of inhibition exhibited by the aqueous extract was 21.5±1.4mm and 20.6±1.09mm against *P. acne* and *S. epidermidis* respectively (Table 1).

Partition coefficient

The partition coefficient (log P) of the aqueous extract was found to be 2.1. This result reveled that both of the extract is appropriate for the development of the topical formulations.

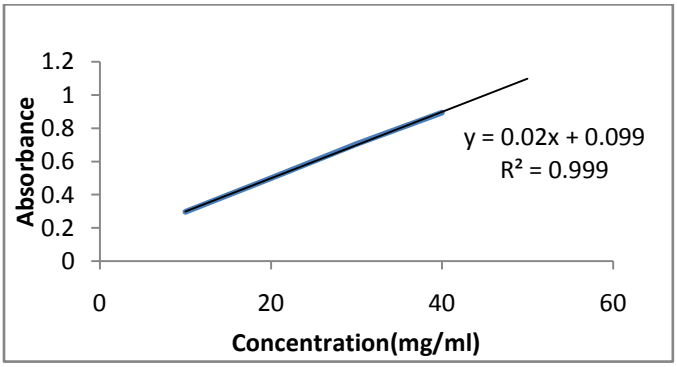

Figure 1: Calibration curve of coriander aqueous extract

Antimicrobial studies of formulations

The antimicrobial activity of the aqueous extract was observed against both the acne producing bacteria. The zone of inhibition was 21.5±1.4mm and 20.6±1.09mm against *P. acne* and *S.epidermidis* respectively. MIC of the extract was found to be 1.7 mg/ml and 2.1 mg/ml against *P. acne* and *S.epidermidis* respectively (Table 1).

Table 1: Antimicrobial activity of coriander extract

S.No.	Test Sample	Zone Of Inhibition (mm)		MIC	
		P. acne	***S. epidermidis***	***P. acne***	***S. epidermidis***
		Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D
1.	Aqueous extract	21.5±1.4	20.6±1.09	1.7 mg/ml	2.1 mg/ml

The formulations were developed by incorporation of penetration enhancer, menthol, in the concentration of 2.5% (Fa_{11}), 5% (Fa_{12}) and 7.5% (Fa_{13}) w/w. The penetration enhancer was incorporated in order to increase the penetration of the formulation through the stratum corneum. These formulations were then evaluated and following observations were noted. The zone of inhibition had also increased against both the acne producing bacterias due to incorporation of penetration enhancer as it was found to increase with increase in the concentration of menthol and exceeded that of the crude extract (Table 2).

Table 2: Composition of formulations

Ingredients	Mass			
	Fa_1	Fa_{11}	Fa_{12}	Fa_{13}
Carbopol 934	0.5	0.5	0.5	0.5
Ethanol	-	15	15	15
Menthol	-	2.5	5	7.5
Methyl paraben	0.15	0.15	0.15	0.15
Propyl paraben	0.03	0.03	0.03	0.03
Propylene glycol	15	15	15	15
PEG 400	5	5	5	5
Triethanolamine	q.s	q.s	q.s	q.s
Distilled Water q.s	100	100	100	100

Physical parameters

The colour of the formulations turned towards white with increase in the content of the penetration enhancer, menthol. All formulations were glossy and produced smooth feel with cooling sensations when applied to the skin.

The pH of the formulations ranged from 6.8 to 7.1, which is suitable for topical application with no discomfort. The viscosities of the formulations ranged from 40.4±0.8 to 37.2±0.5 cps. The decrease in viscosity was due to the presence of ethanol and more over menthol. The viscosity increased with increase in the content of menthol. As the viscosity decreased, spreadibility increased and ranged from 33.3±0.2g/sec to 31.5±0.8g/sec (Table 3). The formulations had neither shown the syneresis at room temperature nor at refrigerated temperatures of 2-8^0 C. The extrudability of the formulation ranged from 539.6±0.8g to 530.2±0.3g. All formulations showed good consistency.

Table 3: Antibacterial activity of formulations

formulations	Zone of inhibition(mm),mean±SD	
	P.acne	***S.epidermidis***
Fa_{11}	21.7±2.4	20.8±2.06
Fa_{12}	21.8±2.0	20.9±2.02
Fa_{13}	21.9±1.9	20.9±2
MH	20.4±.6	20.4±.9
Clin	31.8±2.6	30.9±2.4

All experiments were performed in triplicate. MH = marketed formulation, Clin = Clindamycin phosphate.

Table 4: Evaluation data

formulations	pH[a]	Consistency[a]	Spreadibility(g/sec)[a]	Extrudability(g)[a]	Viscosity(cps)[a]
Fa_{11}	6.9±.05	***	40.4±0.8	539.6±0.2	33.3±0.2
Fa_{12}	7±.08	***	38.5±0.6	534.8±0.6	32.5±0.7
Fa_{13}	6.8±.07	***	37.2±0.5	530.2±0.3	31.5±0.8

a = mean ± standard deviation, *** = very good, ** = good. All experiments were performed in triplicate

Drug content and In-vitro diffusion studies

The drug content ranged from 97.9 to 99.2%. The enhanced drug content was due the presence of ethanol and further the presence of menthol as the drug content increased with increase in the content of menthol. The same pattern was also observed for *in-vitro* diffusion from the formulations. The formulation with 7.5% menthol, Fa_{13}, had highest diffusion rate of 98.9% as compared to the formulation with 2.5% menthol, Fa_{11}, having diffusion rate of 97%(Table 5).

Table 5: Permeation Data of optimized formulations

Form	D.con(%m/m)	%C.rel(diffusion)	%C.rel(perm)	J(µg/cm^{-2}.hr^{-1})[a]	K_p(cm.hr^{-1}.10^{3})[a]	E.R
Fa_1	94.5±2.15	91±1.9	90±1.49	44.6±1.05	1.93±0.01	1
Fa_{11}	97.9±2.6	96.8±2.3	95.8±2.61	74.4±2.09	3.23±0.03	1.6

a = mean ± standard deviation. All experiments were performed in triplicate. Form: Formulations, D.con: Drug content, C.rel: Cumulative release, Perm: permeation, E.R: Enhancement ratio, J: Flux, Kp: permeability coefficient.

Stability studies

During the stability studies, it was observed that formulation Fa_{12} and formulation Fa_{13} had developed a fine crystal layer of precipitated menthol, which might be because of super saturation solubility of menthol in ethanol-water [10]. From the stability studies it was evident that % cumulative drug release and zone of inhibition was decreased for the formulations Fa_{12} and Fa_{13}, which may be due to precipitation on the surface of formulations. Among the formulations of aqueous extract with penetration enhancer, the formulation Fa_{11} was found to be the most stable and thus, the final optimized formulation.

***In-vitro* permeation**

The results of skin permeation studies conducted for 6hrs showed that maximum amount of drug permeated through the skin during 6 hrs was 90% for Fa_1 which rise to 95.8% for Fa_{11} (Table 5). So the permeation of the drug increased after the incorporation of menthol into the formulation of aqueous extract as is also shown in (Figure 2). This precisely showed that the addition of permeation enhancer increases the permeation of the drug through the skin. [11]

The flux was obtained by dividing the cumulative amount of the drug permeated per cm^2 of the skin with time. The flux of the formulations also increased with addition of penetration enhancer, menthol [8, 11] as corresponding flux value increased from 44.6±1.05µg/$cm^{-2}hr^{-1}$ to 74.4±2.09µg/$cm^{-2}hr^{-1}$ for aqueous formulations with and without penetration enhancer respectively. These values of flux indicate that the drugs will dwell at the site of acne lesion and will reach to the follicles.

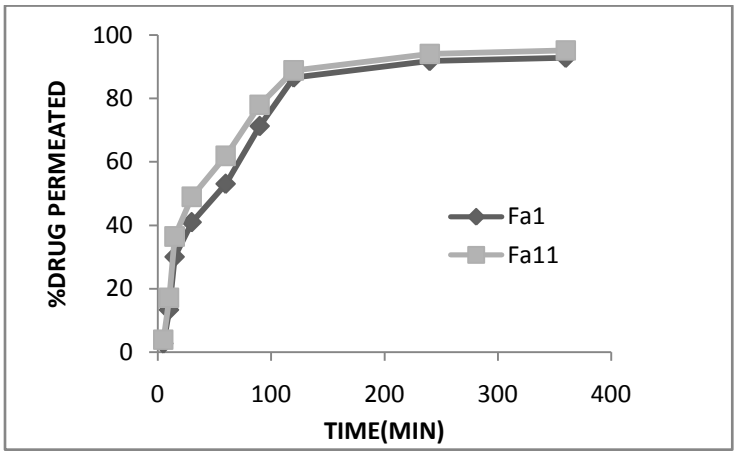

Figure 2: % Cumulative drug permeation for formulations with aq. extract

Release kinetic studies of the optimized formulation

The *in-vitro* diffusion data of the formulations of coriander extract was subjected to kinetic analysis. The selection criteria (R^2) and the equations best describing the kinetics of *in-vitro* drug release are given in Tables 6. The release of the drug from all the formulations was found to follow the first order release kinetics. The results are in agreement with the previous investigation performed by Dua et.al (2011). This implies that the release is concentration dependent. Higher correlation, as indicated by R^2, was observed for the higuchi matrix release kinetics in the optimized formulation (Figure 3), suggesting diffusion as a probable prominent mechanism of drug release. In diffusion, the rate of drug dissolution within the matrix must be much higher than that of the diffusion rate of the drug leaving the matrix. This may be attributed to the nature of gelling agent used.

Table 6: Release Kinetic parameters for optimized formulation.

Form	Zero order model		First order model		Higuchi model	
	R^2	K_0 (min^{-1})	R^2	K_1(min^{-1})	R^2	K_H($min^{-1/2}$)
Fa_{11}	0.9565	0.814	0.9607	0.0038	0.9989	9.9544

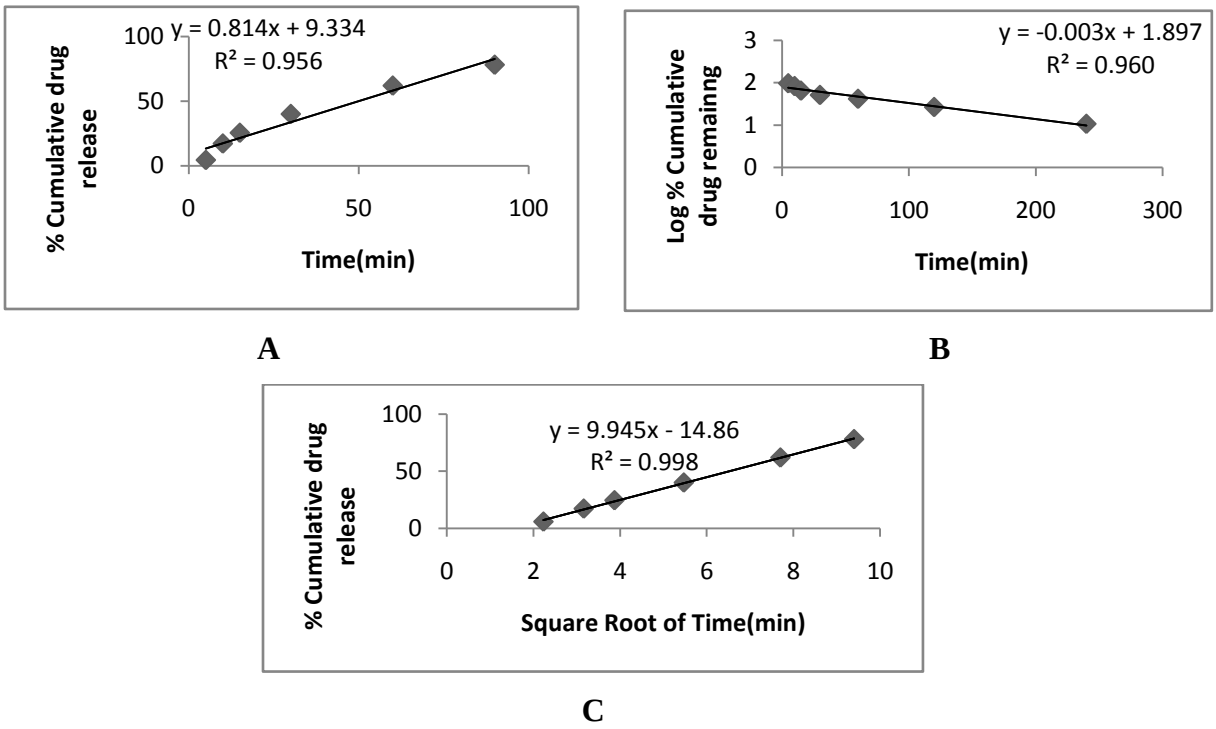

Figure 3: Release Kinetic model for optimized formulation of extract with penetration enhancer.

A: Zero order release kinetic model, B: First order: C: Higuchi model

Rheological evaluation of optimized formulation

The rheological evaluation of the optimized formulation was carried out by hysteresis curve plotting. In this method the shear stress at different speeds in ascending and then in descending order was recorded. The rheogram showed that at the shear rate, the descending curve is plotted under the ascending curve (the shear stress value for recovery is lesser) (Figure 4). This clearly indicates a thixotropic behavior of the formulation. The thixotropic area was calculated by the area between the upward and downward curve. The intrinsic mechanism responsible for conduction of thixotropic behavior majorly relies on three dimensional structures, produced by molecules capable of secondary bonds interactions, which undergo breaking during shear. The viscosity decreases and the gel turning into sol as evident by the ascending curve but when the shearing stress is removed, initial recovery process for the structure is slow(descending curve), the required time for reconstructing by the grouping molecules, that are currently in Brownian movement, depend on applied shear stress.

It was observed from the ascending curve of the rheogram that the formulation showed the non-Newtonian behavior at the work temperature of 37 ^{0} C.

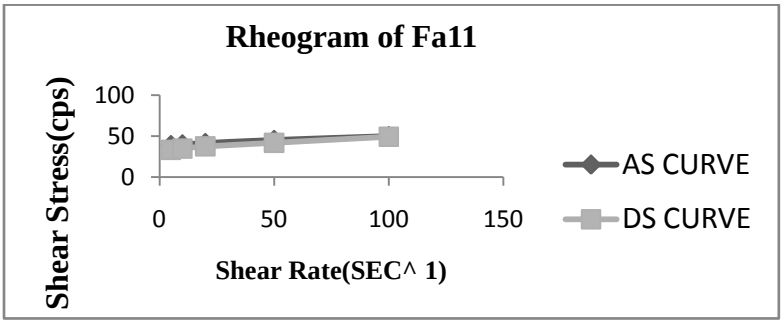

Figure 4: Rheogram of optimized formulation at 37^{0}C.

Interpretation of statistical analysis:

The calculated F values (179 and 195.5) obtained separately from statistical analysis of aqueous extract formulations Fa_1 and Fa_{11} were more than that of table value (4.96) at 5% level of significance. So the null hypothesis was rejected (Table 7). There was significant difference between the % drugs released from the formulations formulated with penetration enhancer

Table 7: Statistical analysis of aq. formulations

Source of information	Sum of squares	Degree of freedom	Mean sum of squares	Calculated F value	Tabulated F value
Between sum of squares	157.56	1	157.5	179	4.97
Error sum of squares	8.79	10	0.879		
Total	166.35	11			

CONCLUSION

The formulation of Coriandrum sativum aqueous extract with menthol, as penetration enhancer was found to have enhanced drug release and antimicrobial activity. This formulation should be tested on human beings and can further be developed with advancement for treatment of acne**.**

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