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Comparative Evaluation of Anti Acne formulations

Aditi Vats[1*]

1.D. J. College of Pharmacy, Niwari Road, Modinagar-201204, U.P.

ABSTRACT

The most common skin disease, acne vulgaris is inflammatory in nature and is caused by bacteria *P. acne* and *S.epidermidis*. The present study was carried out to formulate separately the topical anti acne formulations of coriander aqueous extract and its oil using carbopol as gelling agent and to compare them with marketed formulation. The developed formulations were compared for antimicrobial studies, viscosity and spreadibility, kinetics of release, in-vitro diffusion and permeation. The antibacterial study was conducted by well diffusion method. The permeation of the developed formulations was more than that of marketed one. The marketed formulation was found to be less effective than the coriander formulation.

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

*Corresponding Author Email: sh.aditi.vats@gmail.com

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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 [16,24]. 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 [16]. Thus *P.acne* and *S.epidermidis* are target sites for anti acne drugs.

Acne mainly affects the follicles in the skin so the drug should reach to the dermis and dwell there for considerable period of time. The incorporation of penetration enhancer can serve this motive. Menthol is a well established penetration enhancer with negative heat of sublimation. So, it produces the cooling sensation on application. This is advantageous in case of inflammation [6,8]. *Coriandrum sativum* is medicinally proved to have therapeutic activities like hypoglycemic [21,14], anti-inflammatory [3,4], Hypolipidemic [13,23], antioxidant [12], anti scarring property due to the presence of salicylic acid [8] and anti microbial activity against bacteria and fungi [15]. Coriander mainly comprises of linalool as the major constituent [20]. The coriander with these reported activities can serve as an anti acne agent.

MATERIALS 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

I. **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 20ul 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^{0}C 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[17,19].

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^{0}C 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^{0}C 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. [34, 49, 53]

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[17,19].

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 waterbath 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 in case of coriander aqueous extract.

Preparation of gels

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 continous 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 or Coriander oil dissolved in ethanol) 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.

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 [1,9,11]

Physical parameters

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[5,7].

Viscosity

Viscosities of formulated gels were determined using Brookfield viscometer spindle # 7 at 50 rmps 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[5,7].

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].

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 alluminium 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 [5,7].

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 whish was in horizontal level with the fixed slide. The time taken by the upper slide to slip off the lower slide was noted [5,7].

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

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

Drug Content

The drug content of the gel formulations was determined by dissolving an accurately weighed quantity 0.1gm of gel in 100ml of solvent(phosphate buffer pH 6.8 for formulations of aqueous extract and for marketed formulation while 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 aqueous extract and for marketed formulation while 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 $37^0C \pm 1^0C$. 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 [22].

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 100m 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 1gm of the drug was placed onto the rat skin 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 $37^0C \pm 1^0C$. 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 [25,29].

Stability studies

The stability of the formulations was assessed according to the guide lines issued by International Conference on Harmonization (ICH) on October 27, 1993 [18,26].

Drug release kinetics

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

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

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

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)

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

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).

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

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 [27,28].

RESULT AND DISCUSSION

Antibacterial activity of extract and coriander oil

The extract and oil of coriander were examined for their antibacterial activity against *P.acne* and *S. epidermidis*. The results revealed that both the extract as well as oil inhibit the growth of *P.acne* and *S. epidermidis*. Aqueous extract showed the antibacterial effect with a zone of inhibition of 21.5±1.4 and 20.6±1.09 mm against *P.acne* and *S. epidermidis* respectively (Table 1). The MIC values of 1.7 mg/ml and 2.1 mg/ml were obtained for aqueous extract against *P.acne* and *S. epidermidis* respectively. But the coriander oil showed the greater zone of inhibition (31.4±2.5 and 28.1±2.4mm for *P. acne* and *S.epidermidis*) and lesser MIC (1 and 1.1% against *P.acne* and *S. epidermidis* respectively) as compared to the extract (Table 1).

Table 1: Antimicrobial activity of coriander extract and oil

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
2.	Coriander Oil	31.4±2.5	28.1±2.4	1 %v/v	1.1 %v/v

Partition coefficient

The partition coefficient (log P) of the aqueous extract and oil were found to be 2.1 and 2.93 respectively. This result reveled that both of the extract and the oil are appropriate for the development of the topical formulations.

Antibacterial activity of formulations

The antibacterial activity of all the formulations was detected against acne producing bacteria by

well diffusion method. The zone of inhibition increased against both the bacteria was found to be the maximum for Fo_{11} (31.6±2.2mm and 28.8±2.05mm against *P. acne* and *S.epidermidis* respectively) while that for the marketed formulation was 20.4±.6mm and 20.4±.9mm against *P. acne* and *S.epidermidis* respectively which was lesser than that of Fa_{11} as shown in Table 3. This is attributed to the synergistic anti acne activity of menthol.

Table 2: Composition of formulations

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

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
Fo_{11}	31.6±2.2	28.8±2.05
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.

Physical parameters

Both Fa_{11} and Fo_{11} formulations were glossy and produced smooth feel with cooling sensations when applied to the skin. The MH produced no such cooling effect.

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 were 40.4±0.8, 38.3±0.2 and 46.5±.4 cps for Fa_{11}, Fo_{11} and MH respectively. The lesser viscosity of the developed formulations as compared to the marketed formulation is due to the presence of ethanol and more over menthol. The formulations with less viscosity observed to have more spreadibility (Table 4). The formulations had neither shown the syneresis at room temperature nor at refrigerated temperatures of 2-8^0 C. It was found that all the formulations had shown thixotropic behavior. The extrudability of the formulation ranged from 539.6±0.2, 521.7±0.2 and 552.8±.5g for Fa_{11}, Fo_{11} and MH respectively. All formulations showed good consistency.

Table 4: Evaluation data

Formulations	pH[a]	Consistency	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
Fo_{11}	7.0±.04	***	38.3±0.2	521.7±0.2	32.0±0.5
MH	7.1±.04	**	46.5±.4	552.8±.5	38.4±.4

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

Drug content and *In-vitro* diffusion

The developed formulations were found to have greater drug content (97.9% for Fa_{11} and 98.9% for Fo_{11}) then marketed formulation (88.5%) as shown in Table 4 which shows that there is no loss of the drug during the development process and the method of formulation development is apt for the drug. The drug release after 6 hrs from the diffusion studies was found to be 98.2% for Fo_{11} and 96.8% for Fa_{11} while it was lesser for MH (Table 5). This is due to the presence of menthol in the developed formulations.

Table 5: Drug content and *in-vitro* diffusion release

Formulations	Drug content(%,m/m)	% cumulative release(after 6hrs)
Fa_{11}	97.9	96.8
Fo_{11}	98.9	98.2
MH	88.5	87

All experiments were performed in triplicate

Release kinetic studies

The *in-vitro* diffusion data of the formulations of coriander and marketed formulation 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 Table 6. The release of the drug from developed formulations was found to follow the first order release kinetics which means that the release is the concentration dependent. The results are in agreement with the previous investigation performed by Dua et.al(2011). Higher correlation, as indicated by R^2, was observed for the higuchi matrix release kinetics in the optimized formulation, 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. While the marketed formulation was found to follow the zero order kinetics which implies that the release is concentration independent.

During the stability studies all the formulations were found to be stable.

Table 6: Release Kinetic parameters of formulations.

Form	Zero order model		First order model		Higuchi model	
Fa_{11}	0.9565	0.814	0.9607	0.0038	0.9989	9.9544
Fo_{11}	0.9574	0.7043	0.9602	0.0037	0.999	9.74
MH	0.9843	0.8647	0.9734	0.0042	0.9733	9.12

Permeation studies

The results of in-vitro diffusion studies lead to the conduction of in-vitro permeation studies. The results reveled that maximum amount of drug permeated through the skin during 6 hrs was 97.7, 95.8 and 87% for Fo_{11}, Fa_{11} and MH respectively as shown in Table 7. The permeation of the drug was highest from the oil formulation. This is due to the fact that terpenes present in volatile oil initially have the penetration effect which is further supplemented by the incorporation of menthol. The permeation from marketed formulation was less than that of the aqueous formulation. The flux of the developed formulations (74.4±2.09 $\mu g/cm^{-2}.hr^{-1}$ for Fa_{11} and 85.8±2.2$\mu g/cm^{-2}.hr^{-1}$ for Fo_{11}) is more than that of marketed formulation (39±1.09 $\mu g/cm^{-2}.hr^{-1}$) as evidenced from Table 7. The values of flux are in agreement with fact that the drug will reach to the site of acne and dwell there for considerable period of time which further increases the potency of the formulation in treatment of the disease. The permeability coefficient is also high for these formulations.

Table 7: Permeation Data of formulations

Formulations	% CR	$J(\mu g/cm^{-2}.hr^{-1})^a$	$K_p(cm.hr^{-1}.10^3)^a$	Enhancement ratio
Fa_{11}	95.8	74.4±2.09	3.23±0.03	1.67
Fo_{11}	97.7	85.8±2.2	6.6±0.06	1.73
MH	87	39±1.09	1±0.04	1

a = mean ± standard deviation. All experiments were performed in triplicate.

CONCLUSION

The comparative studies show that the developed formulations of coriander are with greater advantages as compared to the marketed formulation for the better treatment of the disease. Out of the developed formulations the oil formulation is further more active than aqueous formulation. The further clinical studies on human beings can be carried out with these formulations so as develop them at commercial scale.

REFERENCES

1. Vijayalakshmi A, Tripura A, Ravichandiran V. Development and evaluation of anti acne products from *Terminalia arjuna* Bark. Int J PharmTech Res 2011; 3(1):320-327.

2. Ghosh K. V., Nagore H. D., Kadbhune P. K. Different approaches of alternate medicines in acne vulgaris treatment. Orient Pharm Exp Med. 2011;11: 1-9.
3. Wu TT, Tsai CW, Yao HT, Lii CK,Wu YL, Chen PY, Liu KL. Suppressive effects of extracts from the aerial part of Coriandrum sativum on LPS- induced inflammatory responses in murine RAW264.7 macrophages. J Sci Food Agric. 2010;30, 90(11):1846-54.
4. Gupta Sonika, Raghuvanshi Manubala, Jain Deepak. Comparative studies on anti-inflammatory activity of *Coriandrum sativum*, *Datura stramonium* and *Azardirachta indica*. Asian J Exp Biol Sci 2010; 1(1):151-154.
5. Dua Kamal, Pabreja Kavita, Ramana V. M. Aceclofenac topical dosage forms: In vitro and in vivo characterization. Acta Pharmaceutica. 2010; 60:467–478.
6. Patel AN, Patel JN, Patel PR. Formulation and evaluation of curcumin gel for topical application. Pharmaceutical Dev Tech. 2009; 14: 80-89.
7. Das Kuntal, Dang Raman, Machale U Manjunath. Formulation and evaluation of a novel herbal gel of Stevia extract. Iranian J Dermatol. 2009; 12:117-122.
8. Pathan IB, Setty CM. Chemical penetration enhancers for transdermal drug delivery systems. Trop J Pharm Res. 2009; 8: 173-179.
9. Luangnarumitchai S, Lamlertthon S, Tiyaboonchai W. Antimicrobial activity of essential oils against five strains of *Propionibacterium acnes.* J Pharma Sci 2007;34: 60-64.
10. Kumar GS, Jayaveera KN, Kumar Ashok CS, Sanjay P Umachigi et al. Antimicrobial effects of Indian Medicinal Plants against acne inducing bacteria. Trop J Pharma Res 2007; 6(2):717-723.
11. Viyoch J, Pisutthanan N, Faikreua A, Nupangta. K, Wangtorpol K, Ngokkuen J. Evaluation of in-vitro antimicrobial activity of Thai basil oil and their micro-emulsion formulas against Propionibacterium acnes. Int J Cosmetic Sci. 2006; 28: 125-133.
12. Melo A De Enayde, Filho M Jorge. Characterisation of antioxidant compounds in aqueous coriander extract. Lebensm Wiss u Technol. 2005; 38:15-19.
13. Lal AA, Kumar T, Murthy PB, Pillai KS. Hypolipidemic effect of Coriandrum sativum L. in triton-induced hyperlipidemic rats. Indian J Exp Biol. 2004;42 (9):909-912.

14. Gallarher AM, Flatt PR, Duffy G. The effect of traditional antidiabetic plants on in vitro glucose diffusion. Nutrition Res 2003; 23:413-424.
15. Delaquis J. Pascal, Stanich Kareen, Girard Benoit, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J. Food Microbiology. 2002; 7. 101-109.
16. Leyden J James. The evolving role of Propioni bacterium acnes in acne. Seminar Cutaneous Medicine Surgery. 2001;3: 139-143.
17. Andrews M. Jennifer. Determination of minimum inhibitory concentrations. J Antimicrobial Chemotherap. 2001;48. Suppl. S1, 5-16.
18. Carl AB, Edward RA, David EB, Tietz NW (Ed).Textbook of clinical chemistry and molecular diagnostics, 4th rev ed. WB Saunders Philadelphia.2oo1.
19. Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents by agar dilution. EUCAST Definitive Document E. Def 3.1, June 2000.
20. Matasyoh JC, Maiyo ZC. Chemical composition and antimicrobial activity of the essential oil of coriandrum sativum. Food Chemistry. 1999;113:526-529
21. Chithra V, Leelamma S. Coriandrum sativum- mechanism of hypoglycaemic action. Food Chemistry. 1999; 67:229-231.
22. Kobayashi Naoyoshi, Saitoh Izumi. Development of a test method for in-vitro drug release from soluble crystalline dispersion type ointment. Chem Pharm Bull 1999;47: 199-202.
23. Chithra V, Leelamma S. Coriandrum sativum changes the levels of lipid peroxides and activity of antioxidant enzymes in experimental animals. Indian J Biochemistry and Biophysics.1999;36:59-61
24. Stan-posthumud JJ, Vink J, Bruijn JA. Topical tretionin under occlusion on a typical navei. 1998; 548:120-130.
25. Panigarhi L, John T, Shariff A, Shobarani RS. Formulation and evaluation of Lincomycin HCL gels. Indian J Pharma Sci 1997; 59: 610-613.
26. ICH guidelines. Stability testing of new drug substances and products. 27 Oct 1993.
27. Sipahigil Oya, Sahbaz Sevinc, Dortunc Betul, Turkoglu Murat. Diffusion of metronidazole through human skin and synthetic membrane. *Acta Pharmaceutica Sciencia* 2011, 53:99-105.

28. Yasir Mohd, Bajpai Meenakshi, Bhattacharyya Arundhathi. Evaluation of mathematical models describing drug release kinetics from theophylline S.R floating matrix tablets. J Pharm Res 2010,3(9):2265-2269.

29. Clarys Peter, Alewaeters Katrien, Jadoul Ann, Barel Andre, Manadas Oliveira Rui, Preat Veronique,. In-vitro percutaneous penetration through hairless rat skin: influence of temperature, vehicle and penetration enhancers. Eur J Pharma Biopharma1998, 46: 279-283.

30. Blair C, Lewis CA. The pigment of comedones. British J Dermatology, 1970;82: 572-583.