AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and Evaluation of Topical Formulation of Coriander Oil With Penetration Enhancer

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ABSTRACT

Acne is an inflammatory disease of sebaceous follicles of skin. The present study was conducted to formulate and evaluate the topical anti acne formulation of coriander oil with penetration enhancer. The antibacterial activity of Coriander oil against *Propionibacterium acne* and *Staphylococcus epidermidis* was investigated using disc diffusion method and minimum inhibitory concentration was determined by agar dilution method. 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. The results showed that coriander oil showed the MIC values of 1%v/v and 1.1%v/v against *P.acne* and *S. epidermidis* respectively. 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 Fo_{11} showed the drug content (98.9%), *invitro*-diffusion (98.2%) and maximum stability among all the formulations. So it is the potent formulation to be further developed for treatment of acne.

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

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Received 11 August 2012, Accepted 13 August 2012

Please cite this article in press as: Vats A *et al.*, Formulation and Evaluation of Topical Formulation of Coriander Oil With Penetration Enhancer. American Journal of PharmTech Research 2012.

INTRODUCTION

Acne vulgaris is an extremely common disorder affecting virtually all individuals at least once during life. As per global statistics, acne vulgaris affects approximately 85% of population between ages of 12-25 years; nearly 8% adults aged 23-34 years and 3% of adult aged 35-44 years[6, 31]. It is perilous to underestimate its importance as it can have negative psychosocial consequences on the affected individuals including diminished self-esteem, social withdrawl due to embarrassment, depression, unemployment and even suicidal ideations. Acne is an inflammatory disease of sebaceous follicles of skin, marked by comedones, papules, and pustules 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[5]. 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.

Treatment of acne

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. The antibiotics used for the treatment of acne are [13, 31]

Table 1 Agents used for treatment of acne [19,20]

Agents for acne treatment	Application	Adverse effects	Comments
Benzoyl Peroxide	topical	Irritation, bleaching of hair and clothes	
Azelaic acid	topical	Irritation and photosensitivity	
Tetracycline	Topical and oral	Photosensitivity	
Erythromycin	Topical and oral	Gastrointestinal upset	Bacterial resistance
Clindamycin	Topical and oral	Hypersensitivity reaction	Bacterial resistance
Minocycline	Topical	Pigmentation	Expensive

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. [21,19]

This long term expensive treatment increases the instances of alarming adverse effects. The increasing instances of bacterial resistances [24] 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].

According to WHO, it is estimated that at least 25% of all modern medicines were derived either directly or indirectly, from the medicinal plants, primarily through the application of modern technology to traditional knowledge. In case of certain classes of pharmaceuticals, antitumoral and antimicrobial medicines, this percentage may be as high as 60%. [5]

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). This displacement is evident from the increasing room for herbals in their shopping carts. 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].

It is rightly said that "For every disease there is a plant on every continent". Herbal drugs are the best alternatives for the treatment of acne condition as they are safe, dilute, patient familiar, economic, easily available and multifunctional [20]. A number of plants like neem, tulsi, papaya, clove, tree tea oil, etc. have been explored scientifically for their efficacy in treatment of acne as clinical trials had already been conducted [2,4,25]. A number of herbal extracts have already being disguised as selective extracts(employed by reason of their investigation into their specific activity) and a number of them are on their path to this category from the category of total extracts(employed according to historical tradition of their use) [15, 22] as a number of companies are making forays into acne herbal market stating the facts that out of Indian herbal market of Rs 2500 crore, the skin care market is Rs 1200 crore, growing at 20% per annum and herbal acne product market is Rs 65 crore[10]. Still a number of drugs remain in the zone of obscurity. The discoveries of new anti acne herbal drugs expand the herbal anti acne therapeutic and preventive armamentarium.

Coriandrum sativum is medicinally proved to have therapeutic activities like hypoglycemic, [17,28] anti-inflammatory, [7,8] Hypolipidemic, [18,30] antioxidant, [16] anti scarring property due to the presence of salicylic acid [2] and anti microbial activity against bacteria and fungi [23,28].

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.

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 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 (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. [11]

Table 2 :Composition of formulations of coriander oil with penetration enhancer

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

ANTIMICROBIAL STUDIES OF CORIANDER OIL

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 for coriander oil. The

sterile filter paper disc of diameter 6mm were aseptically placed on the inoculated plates and were impregnated with the test material (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^{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. [34,37, 38]

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(0.05- 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, 35, 36]

Minimum bactericidal concentration (MBC) was determined by subculturing 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. [34, 35, 36]

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^{0}C 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 CHARACTERS

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.[33]

Viscosity

Viscosities of formulated gels were determined using Brookfield viscometer spindle # 7 at 50

rmps and 25^{0}C. 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. [33]

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.[10]

Spreadibility

Spreadibility is 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.[12]

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

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(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 (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. [9, 29]

Stability studies

The stability of the formulations was assessed according to the guide lines issued by International Conference on Harmonisation (ICH) on October 27, 1993. [26, 34]

RESULT AND DISCUSSION

The antibacterial activity of Coriander oil 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 oil showed the MIC values of 1%v/v and 1.1%v/v against *P.acne* and *S. epidermidis* respectively(Table 3&4). The formulations were developed by incorporation of penetration enhancer, menthol, in the concentration of 2.5% (Fo_{11}), 5% (Fo_{12}) and 7.5% (Fo_{13}) w/w. Then the formulations were subjected to evaluation and following results were obtained.

Table 3:Antimicrobial activity of coriander oil

S.No.	Test Sample	Zone Of Inhibition (mm)	
		Propionibacterium acne	**Staphylococcus epidermidis**
		Mean±S.D	Mean±S.D
1.	Coriander Oil	31.4±2.5	28.1±2.4

Table 4:MIC and MBC values of coriander oil

S.No	Test Sample	Propionibacterium acne		Staphylococcus epidermidis	
		MIC	MBC	MIC	MBC
1.	Coriander Oil	1 %v/v	1.2 %v/v	1.1 %v/v	1.3 %v/v

Table 5:Antibacterial activity of formulations of Coriander Oil with penetration enhancer.

Formulations	Zone of inhibition(mm),mean±SD	
	P. acne	***S.epidermidis***
Fo_{11}	31.6±2.2	28.8±2.05
Fo_{12}	31.7±2.1	28.9±2.01
Fo_{13}	31.8±2.3	30.1±2.03
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.

The results reveled that the zone of inhibition had also increased against both the acne producing bacterias, P. acne and S.epidermidis, due to incorporation of penetration enhancer as it had increased with increase in the concentration of menthol and had exceeded that that of crude oil (Table 5).

The formulations were of cream to white in colour with characteristic odor of coriander oil. All formulations were glossy and had good consistency.

The pH of the formulations ranged from 6.8 to 7.1, which is suitable for topical application of the formulation with no discomfort. The viscosities of the formulations ranged from 32±0.5 to 30.1±0.2 cps (Table 6). The decrease in viscosity was due to the presence of ethanol and more over due to the presence of menthol. The viscosity decreased with increase in content of menthol.

Table 6:Evaluation data of formulation of coriander oil with penetration enhancer

Formulation	pH[a]	Consistency[a]	Spreadibility(g/sec)[a]	Extrudability(g)[a]	Viscosity(cps)[a]
Fo_{11}	7.0±.04	***	38.3±0.2	521.7±0.2	32.0±0.5
Fo_{12}	6.9±.08	***	35.6±0.8	515.8±0.4	31.2±0.4
Fo_{13}	7.1±.06	***	33.4±0.5	505.4±0.1	30.1±0.2

a = mean ± standard deviation, *** = very good, ** = good.

All experiments were performed in triplicate.

As the viscosity had decreased, the spreadibility was fond to increase and ranged from 38.3±.2 to 33.4±.5g/sec. The extrudability of the formulation ranged from 521.7±0.2 to 505.4±0.1g. All formulations had good consistency. 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 drug content had ranged from 98.9 to 99.6% (Table 7). This is attributed to the cross linkage of carbopol gel and presence of menthol in the formulation along with ethanol. The *invitro* diffusion had ranged from 98.1 to 99.2%. This increase in diffusion is due to menthol present in the formulation and it increased with increase in the concentration of menthol.

Table 7:Drug content and in-vitro-release of formulation of coriander oil with penetration enhancer

Formulations	Drug content(%,m/m)	% Cumulative release
Fo_{11}	98.9	98.2
Fo_{12}	99.2	98.9
Fo_{13}	99.6	99.2

All experiments were performed in triplicate

The formulation Fo_{12} and formulation Fo_{13} had developed a fine crystal layer of menthol during the stability studies. The layer was more prominent in formulation Fo_{13}. The layer might have developed due to saturation solubility of menthol in ethanol- water. But no change was observed in viscosity, spreadibility, extrudability and drug content during stability studies.

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.

From the results it was reveled that formulation Fo_{11} was the most stable and thus the final and optimized formulation among all formulations of coriander oil with penetration enhancer.

CONCLUSION

The formulation of coriander oil with menthol as the penetration enhancer is found to have increased drug release and antimicrobial activity so it can be developed further with advancements for the treatment of acne.

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