FORMULATION AND EVALUATION OF TOPICAL ANTI ACNE FORMULATION OF CORIANDER OIL
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Received 26 August 2012; accepted 07 September 2012

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. The antibacterial activity of Coriander oil 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 topical formulations were developed 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. The formulation F01 showed the maximum drug content (96.5%), in-vitro diffusion (95%) and maximum stability and the zone of inhibition among all the formulations.

KEYWORDS: acne vulgaris, antibacterial activity, coriander, topical gel, in-vitro activity.

1. 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. [4, 27] It is perilous to underestimate its importance as it can have negative psychosocial consequences on the affected individuals including diminished self-esteem, social withdrawal 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.

1.1. Clinical Variants of Acne Vulgaris [17]
- Infantile acne- These are the facial acne seen in infants and are thought to be due to influence of maternal androgens.
- Acne Conglobata-This is a severe form of acne which is a part of follicular triad that includes dissecting cellultes of scalp and hideradenitis suppurativa. It is characterized by acne nodules interconnected sinuses and grouped comedones.
- Acne Eexcoriee- It is the condition in which patients tend to prick and scratch the acne at their skin, leading to exacerbation of the smallest lesions. It is common with adolescent girls and young women.
- Acne Fulminance- These are the rare ulcerative form of acne affecting adolescent boys. It has acute inset and is associated with systematic symptoms of fever, bodyweight loss, arthralgia and myalgia.
- Gram Negative Follcilitis- This generally occurs as a complication of long term antibacterial therapy with sudden onset of multiple pustules frequently localized around perioral and perinasal area and result due to gram-negative organism.
- Steroid Acne- These occur secondary to the corticosteroid therapy or cushing`s syndrome. Clinically rashes often appear as a pustular folliculitis on the back and shoulders rather on face.

Acne is a disease of pilosebaceous glands. Multiple factors are responsible for pathogenesis of acne as sebum, abnormal follicular differentiation, hormones, Propionibacterium acne (P. acne), inflammation and nutrition. Hormones-During puberty, increased dehydrotestosterone (DHT) may lead to hyperkeratinisation by their action on infundibular keratinocytes. The hyperkeratinisation in follicular infundibulum and sebaceous duct is one of the
most crucial events in the development of acne lesions. [8] Sebum-The severity of acne is directly proportional to the sebum production. [27] The secretion of sebum increases due to enlargement of sebaceous glands under the stimulatory action of androgens. The sebum of acne patients is characterized by the presence of lipoperoxides due to peroxidation of squalene and diminished levels of sebum antioxidant vitamin E. [7]

Bacteria- Propionibacterium acne is an anaerobic obligate diphteroid that resides beneath the surface of human skin and populates the androgen stimulated sebaceous follicles. The oxidative stress within the pilosebaceous unit changes the environment from aerobic to anaerobic which is the best suited for this gram positive bacterium. It is implicated in development of inflammatory acne as it activates complement and metabolize sebaceous triglycerides into fatty acids which chemo tactically attract neutrophills. [19] S. epidermidis is aerobic organism involved in superficial infection within sebaceous unit. [15] Thus P. acnee and S.epidermidis are target sites for anti acne drugs.

Inflammation-P.acnee may activate keratinocytes and sebocytes via TLR, CD14 and CD1 molecules. TLR2 is expressed on the surface of macrophages surrounding the pilosebaceous follicles in acne lesions. Activation of TLR2 leads to triggering of transcription factor nuclear factor and thus the production of cytokines which along with IL8 and IL12, released from TLR2 positive monocytes, produce the inflammatory lesions of acne. Inflammatory acne comprises of pustules, papules and nodules. [8] Nutrition-Drinking milk causes a direct rise in IGF-1 through a disproportionate elevation in blood sugar and serum insulin level. High glycemic load foods also cause IGF-1 mediated elevations in DHT. IGF-1 levels during teenage years closely parallel acne activity and are likely synergistic with the steroid hormones. [8, 10] Antibiotics are majorly used for the treatment of acne. 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.[7, 16] This long term expensive treatment increases the instances of alarming adverse effects. The increasing instances of bacterial resistances [9] 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. acnee resistance. Naturally derived compounds, particularly those from herbs have been a good prospect for future development of anti acne products. [25]

Coriandrum sativum is medicinally proved to have therapeutic activities like hypoglycemic, [18] anti-inflammatory, [25] Hypolipidemic, antioxidant, [34] anti scarring property due to the presence of salicylic acid [5] and anti microbial activity against bacteria and fungi. [26]

2. 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.

2.1. Extraction of volatile oil of Coriander
The dried coriander seeds (4 kg) were coarsely powdered and volatile oil was extracted through hydrodistillation by using Clevenger apparatus with a flow rate of 4 ml/min at the temperature of 90°C for 4 hrs. The resulting oil water mixture or distillate obtained in receiver was extracted using diethyl ether as solvent (v/v) and dried over sodium sulphate (anhydrous). The organic layer was concentrated at 20°C for further studies.

2.2. 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. P. acnee 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 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°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°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 S. epidermidis was prepared in nutrient agar medium at 24 hrs under aerobic conditions. Test samples of this aerobic bacterium were incubated at 37°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. [11, 12, 14]

II. 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 P. acnee. The inoculated plates were left until the inoculums had set and then incubated under anaerobic conditions at 37°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 *S. epidermidis* were prepared in nutrient agar medium and incubated for 24 hrs at 37°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.

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.[14, 20, 22]

### 2.3. Preparation of gel of Coriander oil

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 concentration of Coriander oil dissolved in ethanol corresponding to its MIC [11] was also incorporated and finally this mixture 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.

### 2.4. 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.

### 2.5. 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[11].

### 2.6. Viscosity- Viscosities of formulated gels were determined using Brookfield viscometer spindle # 7 at 50 rpm's and 25°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[11].

### 2.7. 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 aluminium 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[6].

#### 2.8. 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 held 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[9].

\[
\text{Spreadibility}= \frac{m}{t} \times l \quad (3.1)
\]

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

#### 2.9. Antimicrobial studies of the formulation- The solution of the gels were prepared using 100mg of gel in 10ml of dimethyl sulfoxide. The anti bacterial activity was tested by well diffusion method. *P. acne* was incubated in ASLA agar medium for 48 hrs under anaerobic conditions and adjusted to yield approximately 1x10⁸ 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°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°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 *S. epidermidis* was prepared in nutrient agar medium at 24 hrs under aerobic conditions. Test samples of this aerobic bacterium were incubated at 37°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].

#### 2.10. 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[21, 29].
buffer pH 6.8 (60:40) for the formulations of Coriander oil with Coriander oil which showed that these were more than 5%. The formulation release studies were plotted in various kinetic models. [30] 2.13. Drug release kinetics- To study the release kinetics of the optimized formulation, the data obtained from invitro release studies were plotted in various kinetic models. [30] 3. RESULT AND DISCUSSION The Coriander oil had shown the zone of inhibition of 31.4±2.5 mm and 28.1± 2.4 mm for P. acne and S. epidermidis respectively. It had shown MIC of 1 and 1.1% v/v for P. acne and S. epidermidis respectively (Table [1]). The formulations were developed with Coriander oil using carbopol 934 as gelling agent in the concentration of 0.5% (Fo1), 1% (Fo2), 1.5% (Fo3) and 2% (Fo4) w/w. These were then evaluated and following observations were made. All the formulations were reddish cream in color and had characteristic odor of coriander oil. The formulations were glossy and translucent. On application to the skin all formulations produced smooth effect. The consistency and homogeneity of all formulations were good. The pH for all formulations ranged from 6.8-7.1, which may be suitable for topical application without discomfort. The gel formulations of coriander oil showed inhibitory effect against both P. acne and S. epidermidis. The diameter of zone of inhibition shown by all the formulations (Table [3]) was similar to that of crude oil which is in agreement with the fact that incorporation of drug into gel base does not decrease its antibacterial activity. [31] The diameter of zone of inhibition of the formulations was more than that of marketed formulation which showed that these were more active against acne inducing bacteria as compared to the marketed formulation. DMSO had shown no activity against both the acne inducing bacteria. The viscosities of the formulations ranged from 33.5± 0.3 to 36.4± 0.5 cps (Table [4]). The viscosities of the formulations increased with increase in the carbopol content. The spreadability and the extrudability of the formulations ranged from 40.8±0.5 to 45.6± 0.6 and 525.1±0.2 to 536.9±0.6 g respectively. The decreased viscosity of the formulations had also been attributed to the presence of ethanol in the formulation. The formulations with the highest viscosity had the minimum spreadability and vice - versa. [24] The drug content of the formulations ranged from 91.2 to 36.4± 0.5% (Fo5) w/w. These formulations were then evaluated and following observations were made. All experiments were performed in triplicate. MH = marketed formulation, Clin = Clindamycin phosphate.

2.11. Drug Content- The drug content of the gel formulations was determined by dissolving an accurately weighed quantity 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.

2.12. 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 cm² and 100m height, having a diffusion area of 3.8 cm². A weighed quantity of formulation equivalent to 1gm of gel in 100ml of solvent (a mixture of ethanol and phosphate buffer pH 6.8 (60:40) for formulations of coriander oil) was continuously stirred and the temperature was maintained at 37°C ±1°C. 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 [6, 23].

2.13. Drug release kinetics- To study the release kinetics of the optimized formulation, the data obtained from invitro release studies were plotted in various kinetic models. [30]

3. RESULT AND DISCUSSION

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Table 4: Evaluation data

<table>
<thead>
<tr>
<th>formulations</th>
<th>pH±</th>
<th>Consistency</th>
<th>Spreadability(g/sec)±</th>
<th>Extrudability(g)±</th>
<th>Viscosity(cps)±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fo1</td>
<td>6.9±0.5</td>
<td>***</td>
<td>40.8±0.5</td>
<td>525.1±0.2</td>
<td>33.5±0.3</td>
</tr>
<tr>
<td>Fo2</td>
<td>7±0.8</td>
<td>**</td>
<td>42.1±0.8</td>
<td>528.5±0.1</td>
<td>34.5±0.4</td>
</tr>
<tr>
<td>Fo3</td>
<td>6.8±0.7</td>
<td>**</td>
<td>43.9±0.4</td>
<td>532.4±0.5</td>
<td>35.7±0.9</td>
</tr>
<tr>
<td>Fo4</td>
<td>7.1±0.4</td>
<td>**</td>
<td>45.2±0.6</td>
<td>536.9±0.6</td>
<td>36.4±0.5</td>
</tr>
</tbody>
</table>

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

Table 5: Drug content and invitro-release data

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Drug content(% m/m)</th>
<th>% Cumulative release</th>
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<tr>
<td>Fo1</td>
<td>96.5</td>
<td>95</td>
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<tr>
<td>Fo2</td>
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<td>93.3</td>
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<tr>
<td>Fo3</td>
<td>92.9</td>
<td>91.8</td>
</tr>
<tr>
<td>Fo4</td>
<td>91.2</td>
<td>90.2</td>
</tr>
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</table>

All experiments were performed in triplicate.

Table 6: Release Kinetic parameters for optimized formulation of coriander oil

<table>
<thead>
<tr>
<th>Form</th>
<th>Zero order model</th>
<th>First order model</th>
<th>Higuchi model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fo4</td>
<td>R² K₀( min-¹)</td>
<td>R² K₁(min⁻¹)</td>
<td>R² K₂(min⁻²)</td>
</tr>
<tr>
<td>0.9525</td>
<td>0.7076</td>
<td>0.9786</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

Fig 1: Release Kinetic model for optimized formulation of coriander Oil.
A: Zero order release kinetic model, B: First order release kinetic model, C: Higuchi model.

found to follow the first order release kinetics. The results are in agreement with the previous investigation performed by Dua et.al (2011). Higher correlation, as indicated by R², was observed for the higuchi matrix release kinetics in the optimized 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.

4. CONCLUSION

The coriander oil was found to have good potency against acne inducing bacteria. The formulations developed from it also showed the same results so it can be further used commercially to develop the anti acne formulation and can be further tested on human beings.

5. ABBREVIATIONS

P. acne Propionibacterium acne
S. epidermidis Staphylococcus epidermidis

6. REFERENCES

[22]. Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents by agar dilution.

Source of support: Nil; Conflict of interest: None declared