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# FORMULATION AND EVALUATION OF HERBAL EMULGEL OF OCIMUM TENUIFLORUM AND MENTHA AREVENSIS LEAVES EXTRACT FOR ANTI-ACNE ACTIVITY

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**ABSTRACT**

Acne is a chronic inflammatory follicular disorder of the skin, occurring in specialized pilosebaceous units on the face. The lack of possible cure and associated disadvantages in allopathic medicines has led to extensive research in natural products with anti acne activity. *Ocimum tenuifloram* (tulasi) and *Mentha arvensis* (mint) were found to be efficacious and cost effective anti acne drugs with least side effects as compared to the synthetic drugs used in the treatment of acne. The present work is aimed to design and develop herbal anti acne topical drug delivery system of combined herbal drugs in the form of emulgel. The effect of emulgel is to be compared with the marketed preparation widely used for acne.

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## INTRODUCTION

Acne is a chronic inflammatory follicular disorder of the skin, occurring in specialized pilosebaceous units on the face. Comedonal bacteria, *"Propionibacterium acnes"*, play an important role in the pathogenesis of acne inflammation by inducing polymorphonuclear leukocytes (PMNL) and monocyte and/or macrophages to produce pro-inflammatory mediators. These organisms produce neutrophil chemotactic factors, which attract neutrophils to release inflammatory mediators such as reactive oxygen species (ROS) and lysosomal enzymes, resulting in disruption of the integrity of the follicular epithelium. Acne vulgaris is a ubiquitous disease characterized by blockage of the sebaceous canal with tightly packed horny cells. This often leads to rupture of the follicle and discharge of its contents into the surrounding tissue, resulting in inflammation.

The lack of possible cure and associated disadvantages in allopathic medicines has led to extensive research in natural products with anti acne activity. *Ocimum tenuifloram* (tulasi) and *Mentha arvensis* (mint) were found to be efficacious and cheap anti acne drugs with least side effects as compared to the synthetic drugs used in the treatment of acne. Therefore these two drugs were selected for the present study. Considering the above justification, the present work is aimed to design and develop herbal anti acne topical drug delivery system of combined herbal drugs in the form of emulgel. The effect of emulgel is to be compared with the marketed preparation widely used for acne. Emulgel (Emulsion in gel) has emerged as one of the useful semisolid drug systems as has been improved the stability of emulsion by incorporating in a gel matrix.

The use of gels has been emerged both in cosmetics and in pharmaceutical industry because of its unique array of features. Despite of providing several benefits, gels faces limitations in delivering hydrophobic drug molecules via skin. Hence in order to overcome this limitation, a recent emulgel approach is being used so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels. The use of gels and emulsions as combined dosage form results into formation of emulgel showing dual release.

The treatment of acne includes many synthetic drugs (like benzoyl peroxide, clindamycin, erythromycin etc,). As the drugs are synthetic, they have their respective side effects. In order to avoid such, treatment with natural products is a better option. The present research focuses on the formulation and development of highly effective, less expensive, easily available herbal emulgel with least side effects for topical use.

## MATERIALS AND METHODS[1-6]

*Ocimum tenuiflorum* was collected from the medicinal garden of Hindu College of Pharmacy, Mentha arvensis was purchased from the vegetable market, Guntur, Span80 was purchased from Loba chemise laboratory reagents and fine chemicals Ltd, Mumbai, India, Tween80 was purchased from Loba chemise laboratory reagents and fine chemicals Ltd, Mumbai, India, Potasium dihydrogen phosphate was purchased from Qualigens, Sodium hydroxide was purchased from Qualigens,Liquid Paraffin was purchased from Qualigens, Agar-Agar powder was purchased from SD fine chemicals and Ethanol was purchased from SD chemicals.

### Plant materials and extract preparation:

*Ocimum tenuiflorum* was collected from the medicinal garden of Hindu College of Pharmacy, Guntur, Andhra Pradesh, Mentha arvensis was purchased from vegetable market, Guntur and both the plants were authenticated by Dr. P.Satyanaraya Raju, Plant taxonomist, Departmrnt of Botany and Microbiology, ANU, Guntur. The foreign, earthy matter and residual matters were removed carefully from the leaves, cleaned and dried in shade. The dried leaves were powdered and used for extraction. The powder was placed in the thimble made with filter paper, was loaded into the main chamber of the soxhlet extractor. The soxhlet extractor was placed into a flask containing extaction solvent until it gets exhausted. The extract was filtered and concentrated using Rota-vacuum evaporator. It was stored at 4-8$^0$ C until use.

## FORMULATION DEVELOPMENT:[7]

The composition of *Ocimum tenuiflorum* and *Mentha arvensis* herbal emulgel formulations was shown in Table below. Different formulations were prepared using different ratios of concentrations of both extracts. The gel bases were prepared by dispersing Na CMC heated purified water (80°C) and the dispersion was cooled and left overnight. The oil phase of the emulsion was prepared by dissolving span 80 in light liquid paraffin while the aqueous phase was prepared by dissolving tween 80 in purified water. *Ocimum tenuiflorum* and *Mentha arvensis* extracts were dissolved in ethanol separately and both the solutions were mixed with the aqueous phase.

Both the oily and aqueous phases were separately heated to 70° to 80°C and then the oily phase was added to the aqueous phase with continuous stirring until cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain the emulgel.

## CHARACTERIZATION OF EMULGEL:

### Physical Examination:

The prepared emulgel formulations are inspected visually for their color, appearance and extrudability and phase separation.

### pH Evaluation:

pH evaluation is the important criterion especially for the topical formulation. The pH of the emulgel should be between 5 and 7 to mimic the skin condition. If the pH of the prepared emulgel is acidic or basic, it may cause irritation to the patient. pH of the prepared emulgel was measured using digital pH meter (ELICO LI 613).1gm of gel was dissolved in 100 ml of distilled water and then dip the glass electrode into an emulgel. The measurement of pH of each formulation was done in triplicate and average values were calculated.

**Spreadability:**

Spreadability denotes the extent of area to which the emulgel readily spreads on application to skin or the affected part. The bioavailability efficiency of an emulgel formulation also depends on its spreading value. The spreadability was expressed in terms of time in seconds taken by two slides to slip off from the emulgel which was placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the spreadability. Two sets of glass slides of standard dimensions were taken. The herbal emulgel formulation was placed over one of the slides. The other slide was placed on the top of the emulgel, such that the emulgel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slide.

100gm weight was placed upon the upper slides so that the emulgel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of emulgel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 10 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.8 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time was taken for calculation.

Spreadability was calculated using formula

$$S = M. L / T$$

Where M= weight tied to upper slide (10g)
L = length of glass slide (6.8cm)
T = Time taken to separate the slides

**Drug Content Determination:**[01]

1 gm each formulation containing approximately 50 mg of extract was taken in a 50 ml volumetric flask and diluted with ethanol and shaken to dissolve the drug in ethanol. The solution was filtered through whatmann filter paper. 0.1 ml of the filtrate was pipetted out and diluted to 10 ml with ethanol. The content of the drug was estimated spectrophotometrically by using standard curve plotted at 366 nm.

**Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor**

***In-Vitro* Release/Permeation Studies**: [01]

*In vitro* release studies were carried out using Franz diffusion cell of 10ml capacity. Egg membrane was isolated and used for the study. Pre-weighed emulgel was spread evenly on the egg membrane. The egg membrane was clamped between donor and receptor compartment. The receptor compartment was filled with 10 ml of pH 6.8 phosphate buffer maintained at 37$^{o}$C and stirred by using magnetic stirrer. 1 ml sample was collected at suitable time intervals (i.e., for every 30mins until complete drug was released) and replaced with fresh buffer. The collected samples were analyzed for drug content by UV –Visible Spectrophotometer at 366nm for tulasi and 510nm for mint respectively.

**Skin Irritation Test:**[01]

0.5 g of the optimized formulation was applied on the hair – free skin of rabbits by uniform spreading over an area of 4 cm$^2$. The skin surface was observed for any visible change such as erythema (redness) after 24, 48 and 72 hours of applying the formulation. The mean erythmal sores are recorded depending on the degree of erythema.

| | |
|---|---|
| No erythema | = 0 |
| Slight erythema (barely perceptible – light pink) | = 1 |
| Moderate erythema (dark pink) | = 2 |
| Moderae to severe erythema (light red) | = 3 |
| Severe erythema (extreme redness) | = 4 |

***In vivo* Studies of Optimized formulation:**[02]

**Study protocol**

| | |
|---|---|
| Study period | : 1 month |
| Plant part used for study | : Dried leaves of Tulasi & Mint |
| Induction model proposed for study | : Acne is induced by *Propionibacterium Acne*. |
| Experimental animals | : Rats (Wister albino) |
| No. of animals required for study | : 15 |

**Experimental Design:**

In the experiment, a total of 15 rats were used. The rats were divided into 3 groups comprising of 5 animals in each group as follows:

**Group I** **:** Control (Glycerin, Topical)
**Group II** **:** Standard drug (Clindamycin, topical)
**Group III** **:** Herbal Emulgel formulation (topical)

**Induction of *Propionibacterium Acne* in Rats:**

Three groups of 15 adult male Wister rats (containing 5 in each group) were anaesthetized with diethyl ether, over the interscapular area and under anesthesia, the hair of rats was shaved. The bacterium was induced through transdermal injection. The acne was identified after 48 hours of induction.

**Stability studies:**[03]

Stability studies were carried out for the optimized formulation (EF3) according to International Conference on Harmonization (ICH) guidelines. Short term accelerated stability studies were carried out for the period of 3 months for the formulations. The samples were stored at different temperature conditions i.e., refrigeration temperature (4-8$^{o}$C), room temperature (25 ±2$^{o}$C) and oven maintained at (45°C ± 2$^{o}$C). The stored formulation was analyzed for visual appearance, clarity, pH, spreadability, viscosity and drug content.

## RESULTS AND DISCUSSION

**Compatibility studies:**

From the FT-IR spectrum of physical mixture of the plant extracts and other ingredients, it was revealed that there are no chemical interactions of plant extracts and other ingredients.

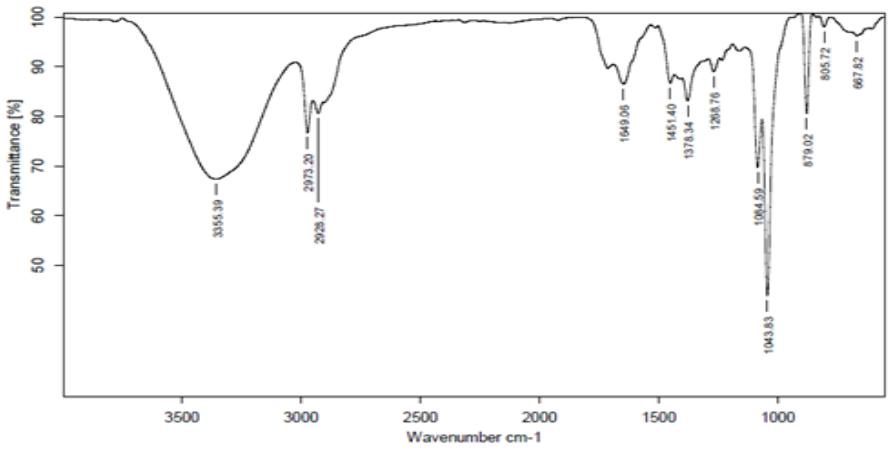


**Fig 1: FT-IR spectrum of ethonolic extract of Tulasi.**

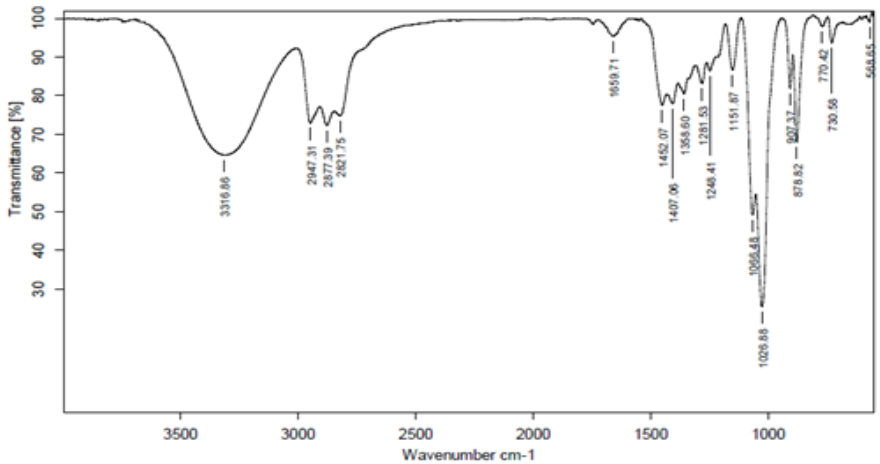


**Fig 2: FT-IR spectrum of ethonolic extract of Mint.**

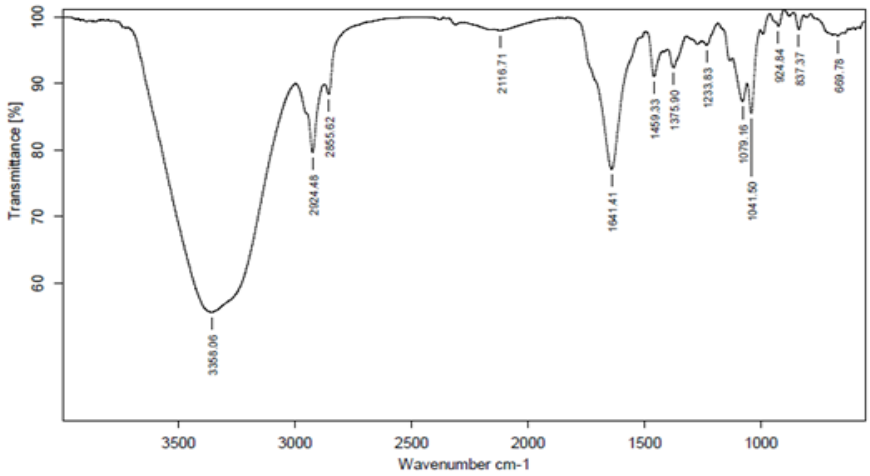


**Fig 3: FT-IR spectrum of optimized formulation EF3.**

**Calibration curve of ethanolic extract of Tulasi in phosphate buffer of pH 5.4:**

Standard plot of *Ocimum tenuiflorum* was plotted as per the procedure in experimental methods and its linearity was shown in Table 2 and Fig 4.The standard graph of *Ocimum tenuiflorum* shows good linearity with $R^2$ value of 0.999,which indicates that it obeys Beer's-Lambert's Law in the concentration range of 0-100 µg/ml.

**Table 1: Calibration curve of ethanolic Tulasi extract in phosphate buffer of pH 5.4.**

| Concentration (µg/ml) | Absorbance(366nm) |
|---|---|
| 0 | 0 |
| 20 | 0.21 |
| 40 | 0.401 |
| 60 | 0.607 |
| 80 | 0.795 |
| 100 | 0.982 |

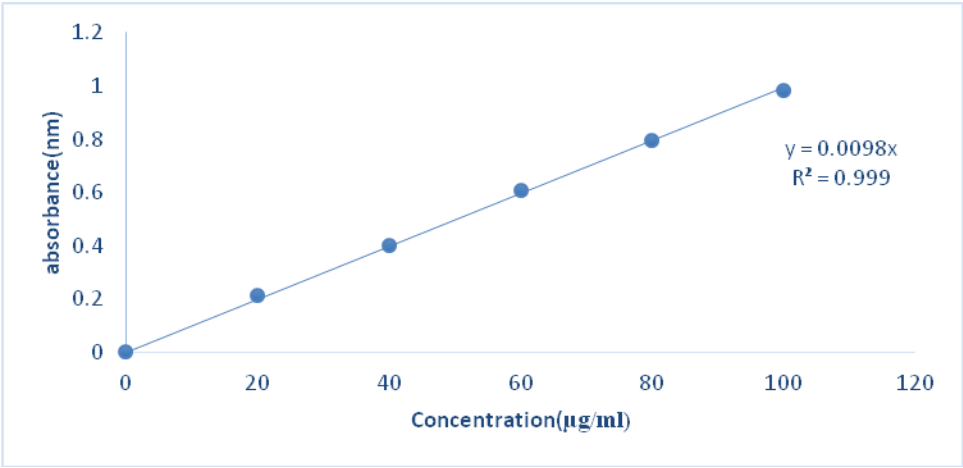


**Fig 4: Calibration curve of ethanolic Tulasi extract in phosphate buffer of pH 5.4.**

**Calibration curve of ethanolic extract of Mint in phosphate buffer of pH 5.4:**

Standard plot of *Mentha arvensis* was plotted as per the procedure in experimental methods and its linearity was shown in Table 3 and Fig 5.The standard graph of *Mentha arvensis* shows good linearity with $R^2$ value of 0.998,which indicates that it obeys Beer's-Lambert's Law in the concentration range of 0-100 µg/ml.

**Table 2: Calibration curve of ethanolic Mint extract in phosphate buffer of pH 5.4.**

| Concentration (µg/ml) | Absorbance(510nm) |
|---|---|
| 0 | 0 |
| 20 | 0.151 |
| 40 | 0.313 |
| 60 | 0.484 |
| 80 | 0.641 |
| 100 | 0.794 |

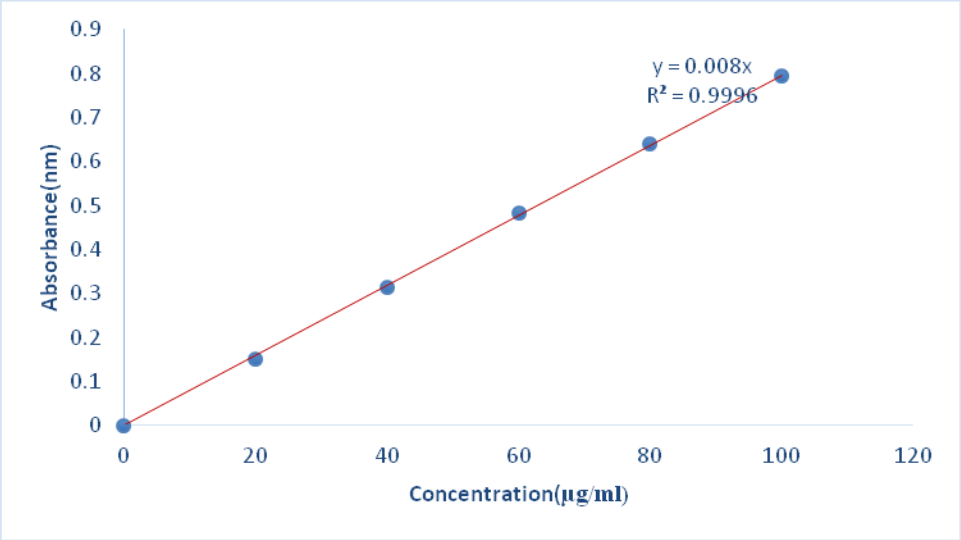


**Fig 5: Calibration curve of ethanolic Mint extract in phosphate buffer of pH 5.4.**

**Physical appearance:**

All the formulations were evaluated for their color and appearance. The physical appearance of all the formulations was found to be greenish, clear and transparent.

**Extrudability studies:**

Extrudability studies of all formulation were carried out as per standards. The results were shown in table 4. It was found that formulations EF1, EF3 and EF4 are having excellent extrudability; Formulations EF2, EF7 were having good extrudability; Formulations EF5 and EF6 were having poor extrudability.

**Table 3: Characterization of emulgel formulations for color, appearance and extrudability.**

| Formulations | Colour | Appearance | Extrudability |
|---|---|---|---|
| EG F1 | Greenish | Clear & Transparent | Excellent |
| EG F2 | Greenish | Clear & Transparent | Good |
| EG F3 | Greenish | Clear & Transparent | Excellent |
| EG F4 | Greenish | Clear & Transparent | Excellent |
| EG F5 | Greenish | Clear & Transparent | Poor |
| EG F6 | Greenish | Clear & Transparent | Poor |
| EG F 7 | Greenish | Clear & Transparent | Good |

**pH determination:**

Skin compatibility is the primary requirement for a good topical formulation. It was found that the pH of all formulations were in the range of pH 5.5 to 7.0 (Table 5) which indicates skin compatibility i.e., emulgels can be applied to the skin without any discomfort or irritation.

**Spreadability studies:**

Spreadability study is one of the criteria for an emulgel to meet the ideal qualities that it should possess good spreadability. If spreadability value is more, it would be properly spread over the skin which is more beneficial as per patient compliance concern.
All the formulations were checked for the spreadability and the data was given in the table 6.9.The values of the spreadability indicated that the emulgels were easily spreadable by small amount of shear.

**Rheological study:**

The viscosities of all the formulations were measured using Brookfield viscometer, spindle 42 (DV++) at 0.5 and 1 rpm and the viscosities for all the formulations were given in the table 5. It was found that all the formulations followed shear thinning effect with thixotropic property.

**Drug content determination:**

Drug content of all the formulations was carried out as per procedure stated in the methodology section. Drug content of all the formulations were found to be in the range of 99-101% as indicated in the table 5. The result indicates that uniform amount of drug is present in all the emulgel formulations.

**Table 4: Characterization of emulgel formulations.**

| Formulation | pH | Spreadability (cm/sec) | Viscosity (cps) | | %Drug content (tulasi) | % Drug content (mint) |
|---|---|---|---|---|---|---|
| | | | 0.5 rpm | 1 rpm | | |
| EF1 | 5.49±0.01 | 17.06±0.11 | 81.1 | 78.3 | 99.92 | 99.81 |
| EF2 | 5.6±0.15 | 17.01±0.32 | 82.7 | 79.1 | 100.22 | 100.06 |
| EF3 | 5.56±0.4 | 17±0.56 | 80.4 | 67.8 | 101.11 | 100.56 |
| EF4 | 5.8±0.1 | 17.03±0.12 | 83.3 | 92 | 99.75 | - |
| EF5 | 5.65±0.2 | 17.02±0.25 | 84.0 | 82.4 | - | 100.97 |
| EF6 | 5.6±0.56 | 17.08±0.54 | 82.5 | 93.5 | 100.51 | - |
| EF7 | 5.9±0.1 | 17.18±0.85 | 82.9 | 91.6 | - | 100 |

***In-vitro* drug permeation for emulgel formulations:**

The emulgel formulations (EF1-EF7) were characterized for their drug diffusion study using Franz diffusion cell through a membrane and the drug content was calculated by simultaneous estimation method. The release studies of Tulasi and Mint from the prepared emulgels were performed in order to study the effect of different types and concentration on the release aiming to select the best formulation.

The release of the tulasi from its emulgel formulations EF1, EF2, EF3, EF4 and EF6 can be ranked in the following descending order: EF3>EF4>EF1>EF2>EF6, whereas the release of Mint from the emulgel formulations EF1, EF2, EF3, EF5 and EF7 can be ranked in the following descending order: EF3>EF1>EF2>EF5>EF7

These results suggested that EGF3 was effective for topical application as highest percentage of drug released after 8hrs (table 6 and 7) in both the cases.

EF3 containing has shown high spreadability (17 cm/sec), less viscosity (80.4 cps), excellent extrudability, more drug content (101%) and highest drug release than all other emulgel formulations. So, it was selected as the optimized formulation.

**Table 5: *in-vitro* drug release profile.**

| Time(min) | Percentage Tulasi released from ethanolic emulgel formulations | | | | |
|---|---|---|---|---|---|
| | EF1 | EF2 | EF3 | EF4 | EF6 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 17.22 | 16.48 | 18.14 | 17.5 | 15.46 |
| 60 | 20.55 | 20.09 | 25.55 | 20.92 | 19.90 |
| 90 | 24.62 | 22.77 | 30.18 | 25.09 | 22.68 |
| 120 | 27.40 | 26.48 | 35.74 | 28.33 | 25.46 |
| 150 | 30.18 | 29.44 | 41.29 | 32.03 | 28.42 |
| 180 | 35.74 | 35.09 | 46.85 | 37.59 | 33.42 |
| 210 | 39.44 | 38.51 | 51.48 | 43.14 | 36.75 |
| 240 | 43.33 | 43.14 | 57.03 | 48.70 | 39.62 |
| 270 | 47.12 | 46.01 | 61.66 | 53.33 | 44.35 |
| 300 | 51.01 | 49.62 | 64.44 | 57.96 | 47.87 |
| 330 | 55.27 | 52.40 | 68.14 | 61.66 | 50.55 |
| 360 | 60.74 | 57.03 | 70.92 | 66.20 | 55.37 |
| 390 | 64.44 | 59.81 | 73.70 | 69.07 | 58.98 |
| 420 | 67.22 | 63.51 | 76.75 | 71.85 | 60.74 |
| 450 | 69.07 | 65.37 | 78.33 | 74.62 | 61.75 |
| 480 | 70.92 | 67.22 | 81.11 | 76.75 | 63.51 |

**Table 6: *in-vitro* drug release profile of emulgel formulations.**

| Time(min) | Percentage Mint released from ethonolic emulgel formulations | | | | |
|---|---|---|---|---|---|
| | EF1 | EF2 | EF3 | EF5 | EF7 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 10.10 | 7.812 | 11.97 | 3.85 | 2.22 |
| 60 | 12.39 | 7.812 | 13.02 | 7.81 | 5 |
| 90 | 15.31 | 10.31 | 15.93 | 11.35 | 6.52 |
| 120 | 15.93 | 13.64 | 16.56 | 12.39 | 6.80 |
| 150 | 16.56 | 14.27 | 23.43 | 13.64 | 7.08 |
| 180 | 20.10 | 17.39 | 29.06 | 14.06 | 7.63 |
| 210 | 26.97 | 18.64 | 37.39 | 17.18 | 8.47 |
| 240 | 37.18 | 24.06 | 46.97 | 18.64 | 9.30 |
| 270 | 42.39 | 29.89 | 55.72 | 26.97 | 13.61 |
| 300 | 54.27 | 36.97 | 70.10 | 33.43 | 18.61 |
| 330 | 56.77 | 43.64 | 83.020 | 42.81 | 22.08 |
| 360 | 58.22 | 49.68 | 84.68 | 47.39 | 28.19 |
| 390 | 62.39 | 55.93 | 89.47 | 51.77 | 30.27 |
| 420 | 76.97 | 60.31 | 93.43 | 53.85 | 32.22 |
| 450 | 85.31 | 71.97 | 95.93 | 58.22 | 34.16 |
| 480 | 92.81 | 79.06 | 96.56 | 71.97 | 40 |

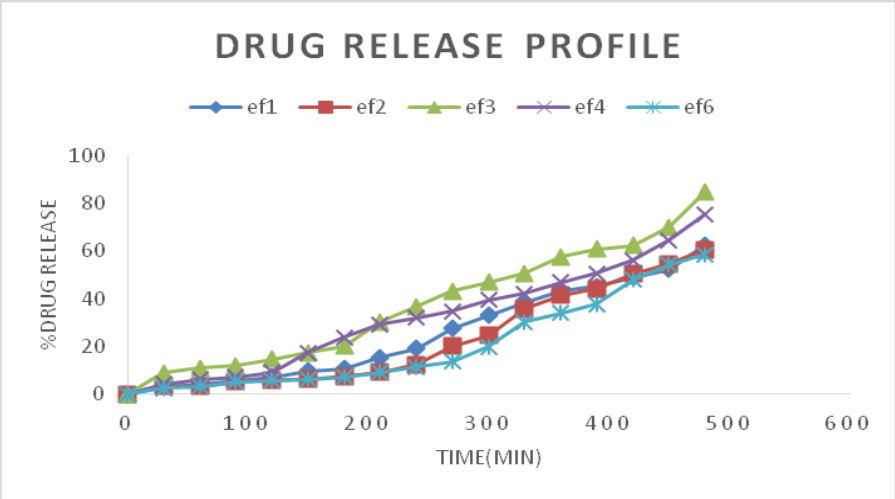


**Fig 6: Drug release profiles of emulgel formulations containing Tulasi.**

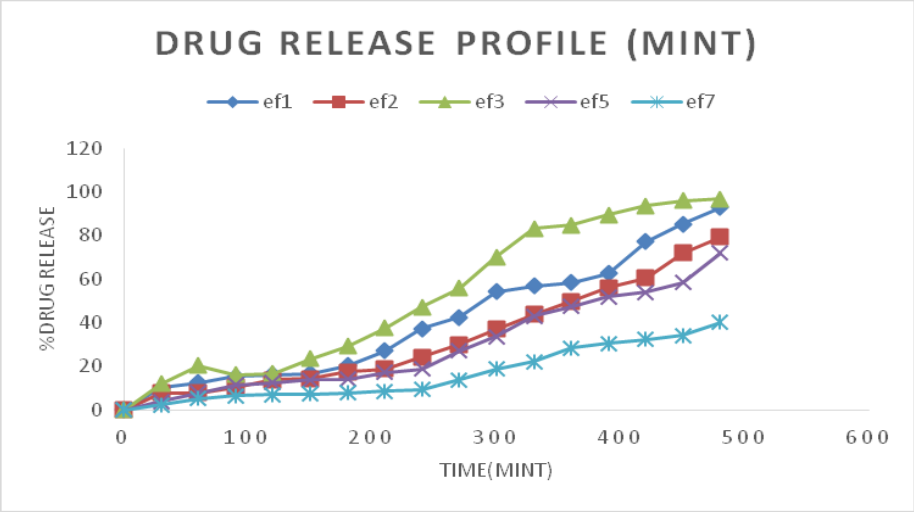


**Fig 7: Drug release profiles of emulgel formulations containing Mint.**

**Release mechanisms**

By incorporating release data in Higuchi and erosion models, the $R^2$ values of all the formulations were found to be greater for Higuchi model. So, all the formulations in this study were best expressed by Higuchi's classical diffusion equation. The linearity of plot indicated that the release process was diffusion controlled.

To further confirm the exact mechanism of drug release, the data was incorporated into koresmeyer Peppas model and the mechanism of drug release was indicated according to the value of exponent 'n'. For all the emulgel formulations the release exponent 'n' value found to be between 0.696 and 0.739. This indicates the drug released from all the emulgel formulations followed non – fickian diffusion mechanism.

**Table 7: correlation coefficient values of emulgel formulations containing Tulasi.**

| Formulations | Zero order($R^2$) | First order($R^2$) | Higuchi ($R^2$) | Erosion ($R^2$) | Korseymeyer peppas | |
|---|---|---|---|---|---|---|
| | | | | | $R^2$ | n |
| EF3 | 0.975 | 0.862 | 0.869 | 0.747 | 0.943 | 0.713 |

**Table 8: correlation coefficient values of emulgel formulations containing Mint.**

| Formulations | Zero order($R^2$) | First order($R^2$) | Higuchi ($R^2$) | Erosion ($R^2$) | Korseymeyer peppas | |
|---|---|---|---|---|---|---|
| | | | | | $R^2$ | n |
| EF3 | 0.961 | 0.882 | 0.867 | 0.784 | 0.945 | 0.754 |

**Skin Irritation Test:**

The optimized formulation EGF2 was applied on the hair free skin of the rabbit. The applied area is observed for 3 days and the result shows that the primary irritation index of the sample was 0.00 i.e., no erythema, edema and no irritation were observed on the skin of the rabbit. The formulation is safe for topical use.

**In-Vivo animal studies:**

The animal activity was started after getting IAEC permission (HCOP/IAEC/PR-7/2018). The weight of the rats was measured prior to bacterium induction. The effect of ethanolic extract of leaves of Tulasi and Mint emulgel on rats was evaluated, the acne healing contracting ability of standard and test were significantly greater than that of control.

Test rats (which received optimized formulation) showed comparable healing activity to that of standard rats (which received clindamycin ointment).

It was further found that all the three groups showed decreasing acne from day to day.

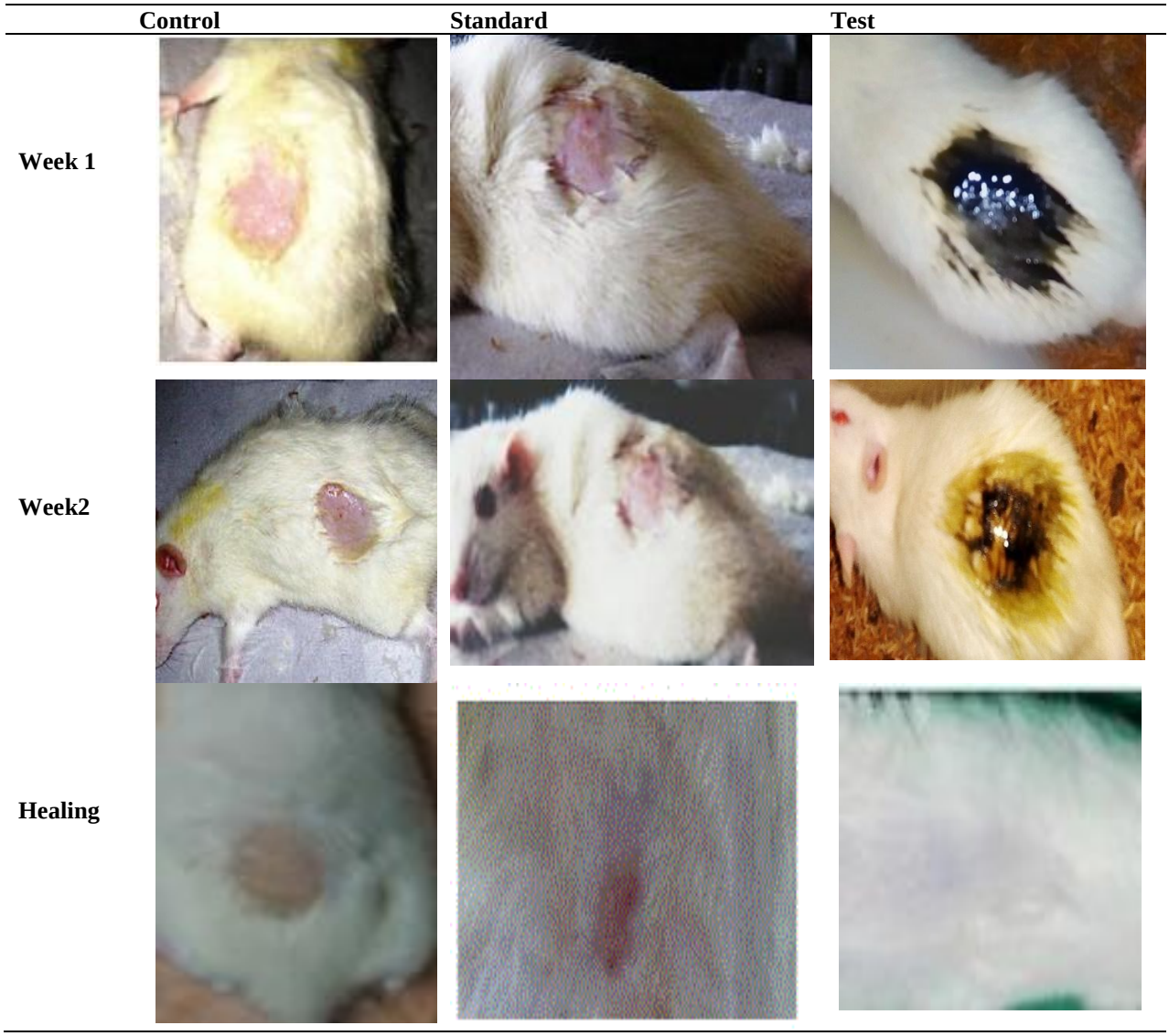


**Fig 8 : Anti-acne activity images.**

**Stability studies:**

Stability study was performed on optimized batch EF3 at ambient conditions. The results obtained after 3 months time period were shown in the table. The results revealed that there is no significant change in pH, color and drug content in optimized formulation. The results conclude that the optimized formulation is stable for 3 months and can be applied topically.

**Table 9: Short term stability data of optimized formulation.**

| Initial | | After 3 months |
|---|---|---|
| **Colour** | **pH** | **Drug content (%)** |
| Greenish | 5.42 | 99±0.21 |

**DISCUSSION**

In this study the topical application of the optimized formulation on the infected area of rats caused a significant and faster healing. Although acne treatment with emulgel containing the extracts of Tulasi and mint showed anti-acne activity, the exact mechanism in acne healing was not established.

Further phytochemical studies are needed to isolate the active compounds reasonable for anti-acne activity. Further studies with purified constituents are needed to understand the complete mechanism of anti-acne activity of Tulasi and Mint leaves extract.

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