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Research Article

# Tulsi Oil Loaded Biocompatible, Stable Organogel with Improved Physical Stability and Prolonged Activity.

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**ABSTRACT**

Tulsi oil, though reported to be affective against broad spectrum bacteria and fungi the simple conventional gel formulations could not enter the clinical trials due to instability of Tulsi oil. Microbial growth in the formulation, phase separation and incompatibility with aqueous components are the major concerns associated with formulation and development involving Tulsi oil as the active component. In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils and Eugenol in therapeutic potentials of *Ocimum sanctum Linn.* Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsi plant. Various workers have tried extract of *Ocimum sanctum* against some well known fungal etiological agents as *Candida albicans*. Candidiasis is a very common disease not only in human but also in animals and therefore always has been a challenge to scientist. Overgrowth of several species including *albicans* can cause superficial infections such as oropharyngealcandidiasis (thrush) and vulvo vaginal candidiasis (vaginal candidiasis). Oral candidiasis is common in elderly denture wearers. In otherwise healthy individuals, these infections can be cured with topical or systemic antifungal medications (commonly over-the-counter antifungal treatments like miconazole or clotrimazole). Ointments, creams, liquid preparations, powders, aerosols, gels are various topical drug delivery systems. A ternary plot depicting proportions of water, Tulsi oil and surfactant mixture with gelator, was prepared to predict the gelling compositions. To figure out the area of ternary plot fulfilling MIC, antifungal susceptibility testing of Tulsi oil was carried out. *In- vitro* diffusion study was done and percent release was quantified in terms of Eugenol. Further Stability studies were performed. HET- CAM test was carried out to assess biocompatibility. The drug release was found to follow Korsmeyer-peppas model with zero order release pattern which was supported by higher release exponent value, indicating super case II transport systems. The optimized organogel showed shelf life of 54, 42, and 22 days at $4^0$C, $25^0$C, $50^0$C respectively and was found to be biocompatible and physically stable for longer period as compared to conventional Tulsi oil gel formulation. The present study highlights the usefulness of approach selected for study to improve the formulation aspects in terms of physical stability of oily active components.

**Keywords:** Tulsi oil, Eugenol, Organogel, Stability study

## INTRODUCTION

Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family Labiatae are very important for their therapeutic potentials. Aqueous decoction of Tulsi leaves is given to patients suffering from gastric and hepatic disorders. Herbal preparations containing *Ocimum sanctum L.* have been suggested to shorten the course of illness, clinical symptoms and biochemical parameters in patients suffering from viral hepatitis[1]. Paste of Tulsi leaves is found to be effective in the treatment of ring-worm and other skin diseases. The seeds of *Ocimummericanum*have been found to possess antifertility activity in women when given along with *Actinopteris radiate*. Ursolic acid, one of the major constituents of the Tulsi leaves, has been suggested to possess antifertility effect in rats of both sexes and in male mice. Ursolic acid because of its anti-estrogenic effect reduces spermatogenesis and causes a decrease in sperm counts. Several other pharmacological effects, such as antitumor, hepato protective, anti-inflammatory (oral & topical), anti-ulcer, antimicrobial, anti-hyperlipidemic`, and anti-viral activities, have also been attributed to ursolic acid[2]. Various workers have tried extract of Ocimum sanctum against some well known fungal etiological agents as *Candida albicans* Candidiasis is a very common disease not only in human but also in animals and therefore always has been a challenge to scientist. Ethanolic extracts of *O. sanctum* (whole plant) were reported to have 21-30mm zone of inhibition against Candida albicans and were less effective in comparison to aqueous extract in contrast to these there are also some reports with almost negligible effects of chloroform, acetone and Methanolic extracts of *O. basilicum L.* against *Candida albicans*

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Table 1: Coded and actual values of Ingredient

| Independent variable ($X_1$, $X_2$) | Actual Values in gm Low level | High level |
|---|---|---|
| $X_1$ | 50 | 80 |

Table 2: Formulation as Per $2^2$ Full Factorial Design Layouts, Experimental runs and their factor combination

| S. No | Ingredients (gm) | A | B | C | D |
|---|---|---|---|---|---|
| 1. | Tulsi oil | 20 | 20 | 20 | 20 |
| 2. | Surfactant | 80 | 50 | 80 | 50 |
| 2A | Span 80 | 40 | 25 | 40 | 25 |
| 2B | Tween 80 | 40 | 25 | 40 | 25 |
| 3. | Carbopol 940 | 0.5 | 0.5 | 0.5 | 0.5 |
| 4. | Water | 30 | 0 | 0 | 30 |
| 5. | Methyl paraben | 0.03 | 0.03 | 0.03 | 0.03 |
| 6. | Propyl paraben | 0.01 | 0.01 | 0.01 | 0.01 |
| 7. | NaOH (0.1N) | q.s | q.s. | q.s. | q.s. |

Table 3: Composition of Optimized Batch Organogel

| S. No | Ingredients | Concentration (gm) |
|---|---|---|
| 1. | Tulsi oil | 20 |
| 2. | Surfactant | 55.88 |
| 2A | Span 80 | 27.94 |
| 2B | Tween 80 | 27.94 |
| 3. | Carbopol 940 | 0.5 |
| 4. | Water | 26.75 |
| 5. | Methyl paraben | 0.03 |
| 6. | Propyl paraben | 0.01 |
| 7. | NaOH (0.1N) | q.s |

Table 4: Schoring Scheme for Evaluation of Test Results

| | score | | |
|---|---|---|---|
| Effect | 0.5 min. | 2 min. | 5 min. |
| Lysis | 5 | 3 | 1 |
| Haemorrhage | 7 | 5 | 3 |

Table 5: Composition of Optimized Batch Hydrogel

| S. No | Ingredients | Concentration Gm |
|---|---|---|
| 1. | Tulsi oil | 20 |
| 2. | Surfactant | 55.88 |
| 2A | Span 80 | 27.94 |
| 2B | Tween 80 | 27.94 |
| 3. | Carbopol 940 | 0.5 |
| 4. | Water | q.s |
| 5. | Methyl paraben | 0.03 |
| 6. | Propyl paraben | 0.01 |
| 7. | NaOH (0.1N) | q.s |

ATCC 845981, Candida crusei ATCC 6258, *Candida albicans* ATCC 90028[1]. The therapeutic potential of the essential oils extracted from fresh leaves of *Ocimum sanctum L.* has been found to be largely due to Eugenol (major constituent of the essential oil) which is a phenolic compound (l-hydroxy-2-methoxy-4-allyl benzene). In order to understand the mode of action of *Ocimum*, therapeutic uses of *Ocimum Sanctum Linn* (Tulsi) *sanctum L.*, to explain its therapeutic potentials in management of various disease conditions and to establish its use in modern medicine, several investigations have been carried out to study the pharmacological actions of the Eugenol, essential oils (extracted from Tulsi leaves) and extracts of Tulsi on immune system, central nervous system, gastric system, reproductive system, blood biochemistry etc. in experimental animals. Essential oils extracted from the leaves of *Ocimum sanctum L.* has been found to inhibit *in-vitro* growth of *E. coli*, *B. anthracis*and, *P. aeruginosa*showing its antibacterial activity. Tulsi also has anti-tubercular activity and inhibits *in-vitro* growth of *M. tuberculosis*. The essential oils extracted from Tulsi leaves also possess anti-fungal and anti-viral activity[1]. Topical delivery can be defined as the application of the drug containing formulation to skin to directly treat coetaneous disorders (psoriasis and acne etc) with the intent of containing the pharmacological or there effect of the drug to surface of skin or within. There are two basic types of drug delivery products, external topical and internal topical. The external topical are spread, sprayed or otherwise dispersed on the tissue to cover the diseased area, while the internal topical are applied to mucous membrane orally, vaginally, or on the rectal tissues for local activity. Advantages of Topical Drug Delivery Systems are avoidance of first pass metabolism, convenient and easy to apply, Achievement of efficacy with lower total daily dosage of drug by continuous drug input[3,4]. The delivery of drug via transdermal route has been recognized as one of the potential routes for both local and systemic delivery of drugs, due to several advantages. Topical delivery of bioactive substances is indeed a powerful strategy to reduce their systemic toxicity and at the same time restricts the therapeutic effect to specific tissues targeting to a specific site[5]. Biological factors like skin conditions, skin sage, blood flow, regional skin sites, skin Metabolism and physicochemical Factors like skin Hydration, Temperature and pH, diffusion coefficient, drug concentration, partition coefficient, molecular size affects Absorption of Drug through Skin. Various Topical Drug Delivery Systems are ointments, creams, liquid preparation, powders, aerosols, gels[6]. Gel is defined as semisolid preparation; consist of dispersion of small & large molecule in aqueous vehicle rendered jelly-like through the addition of gelling agent. Classification of Gel

- Primary Types
  - Hydrogel
  - Organogel
- Based on Nature of Bond
  - Chemical Gel
  - Physical Gel

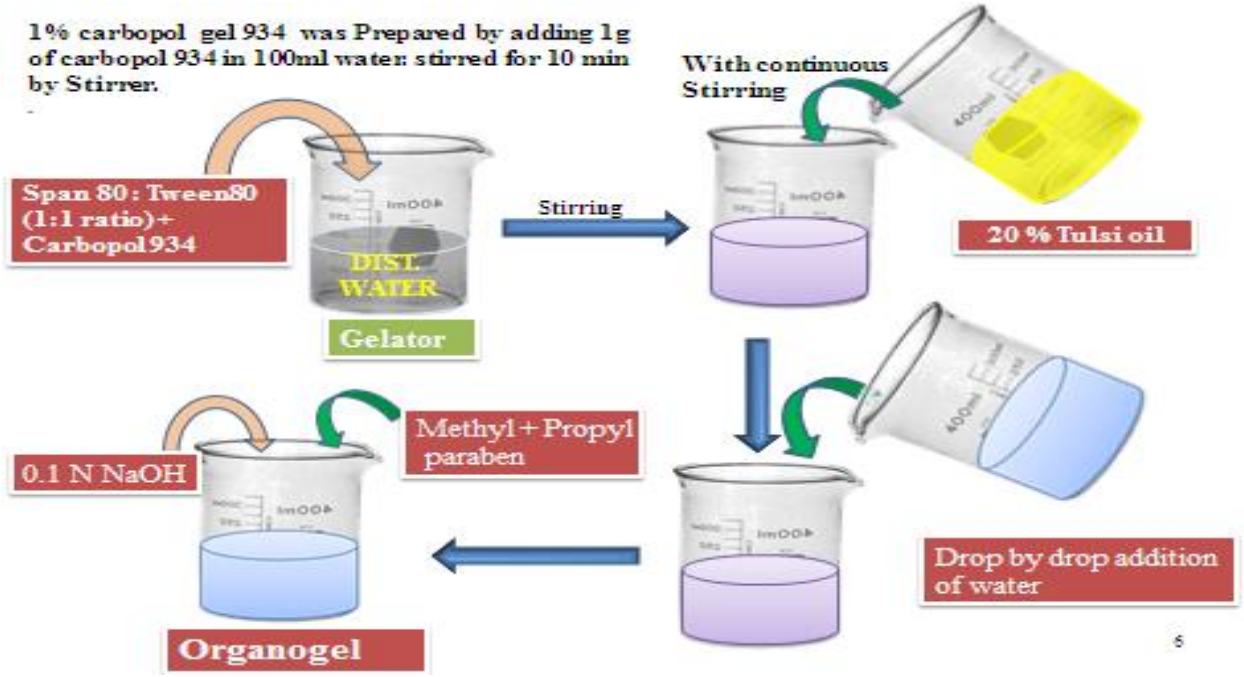


Figure 1: Procedure for Preparation of Organogel

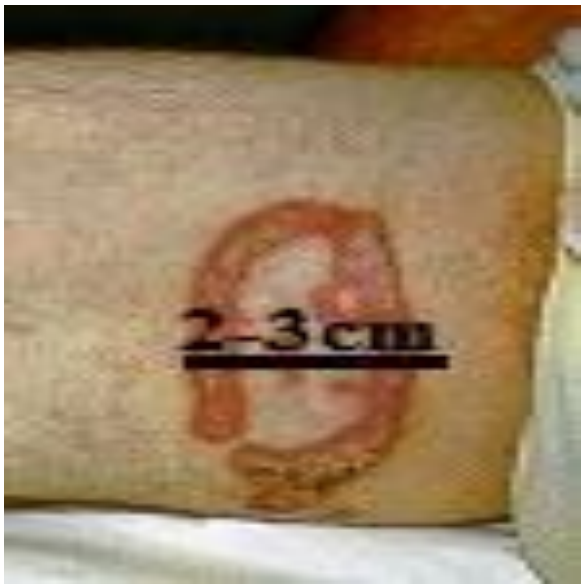


Figure 2: Area of Ringworm Infection

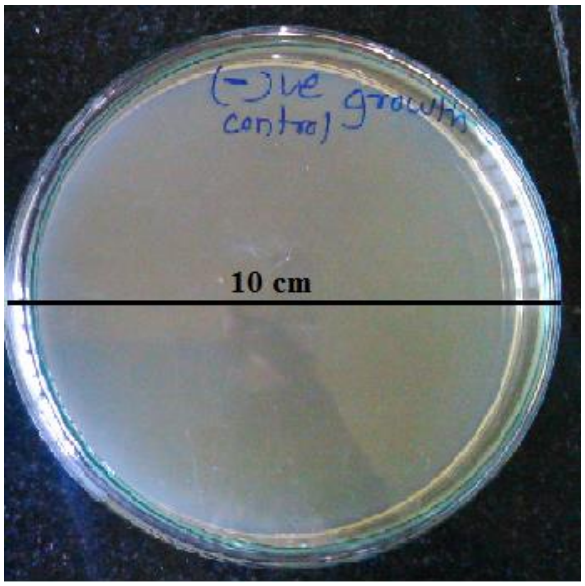


Figure 3: Area of Petriplate

➢ Depend on phase System
- Two Phase System
- Single Phase System

Organogels are highly self-structured system, which are isotopic, thermo reversible, semi rigid system formed by peculiar kinds of small organic molecules. Organogel consist of macromolecules existing as twisted strands therefore do not form semisolid on standing. The molecules are bound together by stronger types of vander-waals forces so as to form crystalline amorphous region throughout the entire system[7]. Advantages are not necessary to add penetration enhancer hence less harmful, not produce irritation, Moisture insensitive, less resistance to the microbial contamination. While disadvantages are less stable to temperature, Organogel naturally shrink when kept for longer time, Drug having high liphophilicity and partition coefficient can be used, Stability problem

*Classification of Organogel*

1.LecithinOrganogel 2. Microemulsion Based Organogel 3. Gelatin stabilized microemulsion based organogel 4. Premium Lecithin Organogel. 5. Pluronic Lecithin Organogel[8-10].

Factors Affecting Organogel is polar solvent, non-aqueous solvent, phase transition temperature (PTT), salt addition, temperature, surfactants Applications are parentraldelivery, oral delivery, topical/transdermal delivery Thus, from the present literature review the major point to be highlighted is unavailability of stable dosage form of Tulsi oil in terms of physical and chemical stability. The aim of present research work would be to

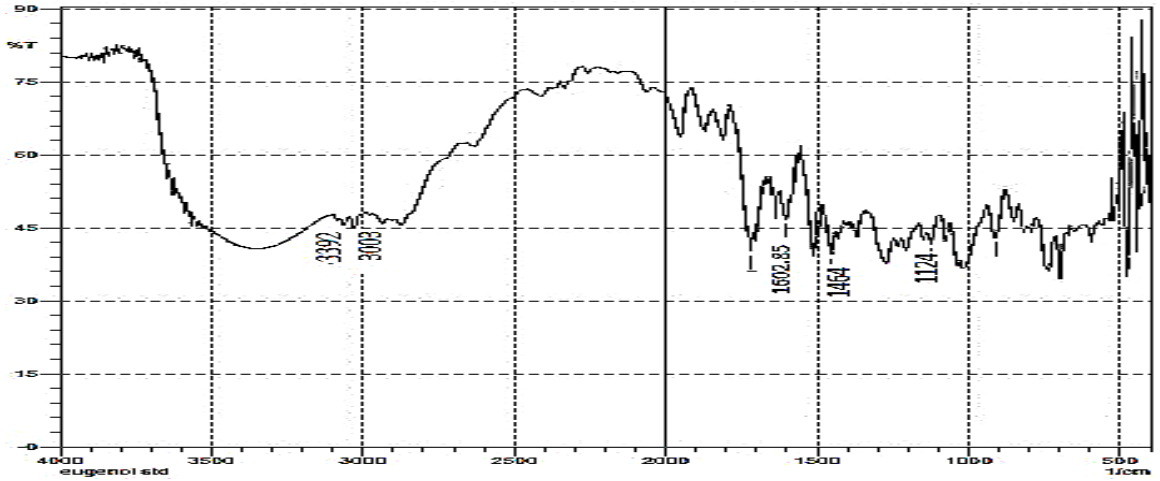


Figure 4: FTIR Spectra of Eugenol

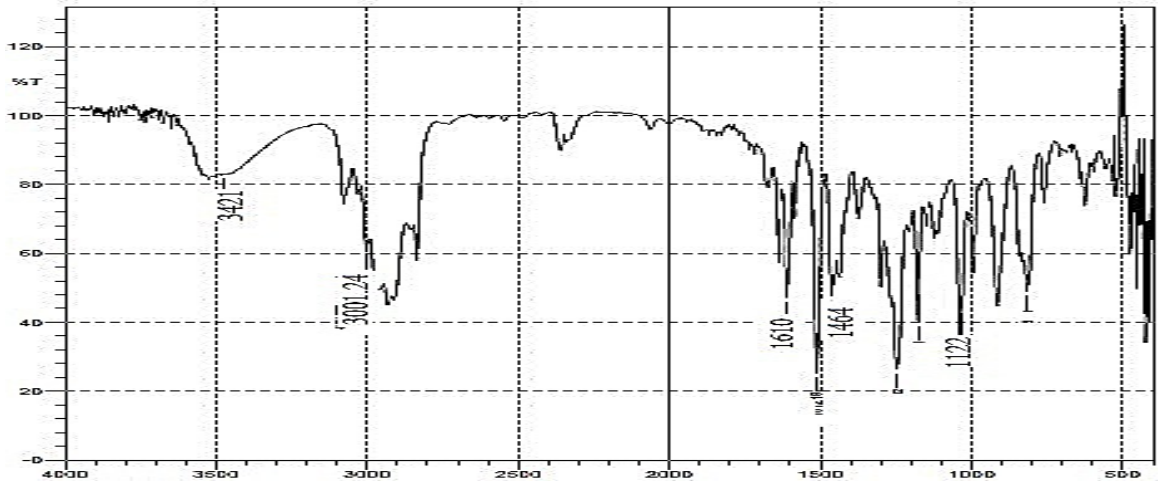


Figure 5: FTIR Spectra of Tulsi oil

Table 10: The absorbance of Tulsi oil in Methanol

| Conc(ppm) | Absorbance (281 nm) |
|---|---|
| 0.2 | 0.1634 |
| 0.4 | 0.2960 |
| 0.6 | 0.3575 |
| 0.8 | 0.4393 |
| 1 | 0.6689 |

focus on development of organogel formulation of Tulsi oil with improved stability and applications in turn.

**MATERIALS**

Eugenol marker sample was procured from Bansal Sales, Nashik. (CAS No. 97-53-0). Tulsi oil was procured from local market. Caropol 934, span 80 and Tween 80 were procured from LOBA Chemie. Methyl paraben, propyl paraben and NaOH were procured from Thomas Baker, Mumbai while, SDA and Saboured Dextrose Broth were procured from Himedia Laboratories, Pvt, Ltd, Mumbai.

Experimental

*Characterization of Tulsi Oil*

*FTIR Spectroscopy*

*FTIR Spectroscopy of Eugenol Marker Sample and Tulsi oil*

The IR spectra of was recorded using fourier transform infra-red spectrophotometer (SHIMADZU, Japan) with diffuse reflectance principle. Sample preparation kept between potassium bromide (KBr) cells, finally placing in the sample holder. The spectrum was scanned over a frequency range 4000 – 400 $cm^{-1}$. Results off Eugenol are mentioned in Fig.4 and while, results of Tulsi oil are mentioned in Fig.5.

*Boiling Point*

The boiling point of Tulsi oil was determined by using Thiele's tube method. The substance under test was filled into sodium fusion tube. Liquid paraffin bath was heated and sodium fusion tube tied to thermometer. The temperature at which the substance boiled was recorded as a boiling point of the substance. This study was performed in triplicate. Results are discussed in result and discussion section.

*Construction of Calibration Curves*

Eugenol marker sample was used to construct calibration curve.

*Determination of wavelength maximum*

*Determination of wavelength maximum in Methanol and Phosphate Buffer (pH 7.4)*

The standard solution of Eugenol marker was prepared in methanol and Phosphate Buffer (pH 7.4). The prepared solution was scanned between in the range of 400-200 nm by UV- visible spectrophotometer (Jasco V-630, Japan).

*Content Estimation of Eugenol from Tulsi Oil*[11].

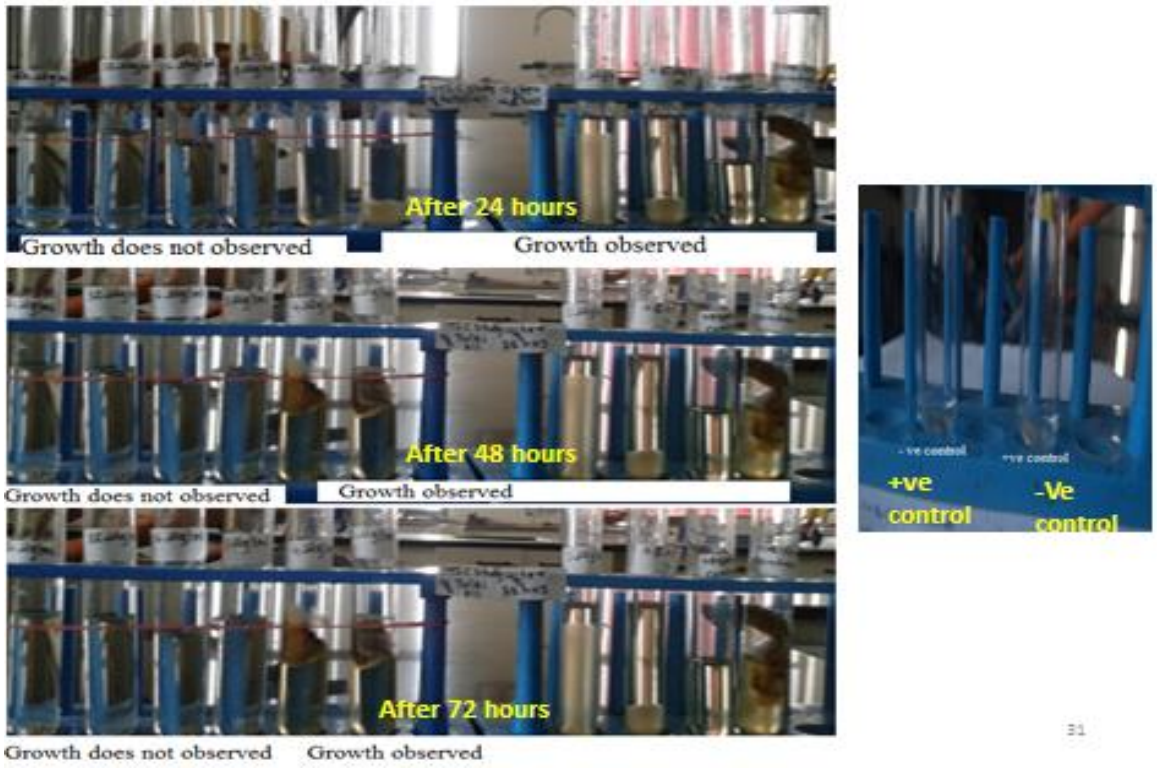


Figure 6: Minimum Inhibitory Concentration (MIC) of Eugenol (Tulsi Oil)

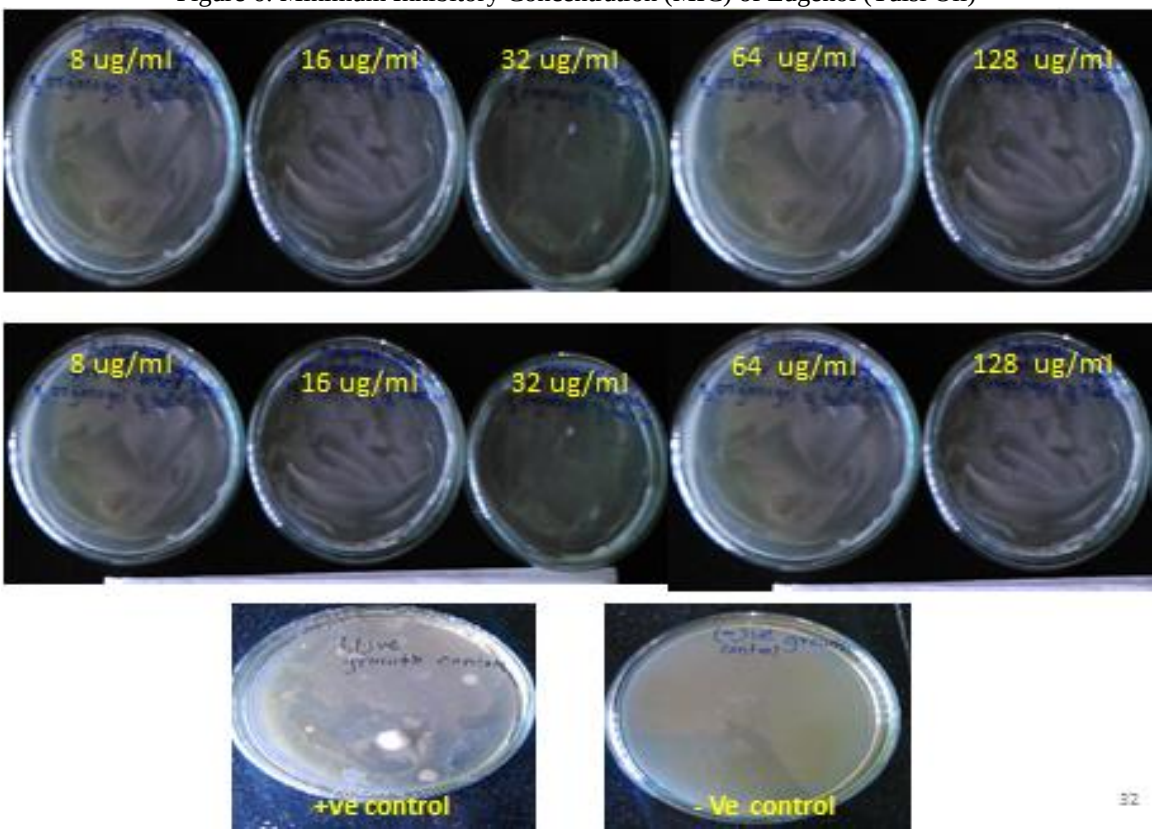


Figure 7: Minimum Fungicidal Concentration (MFC) in Eugenol (Tulsi Oil)

- From reported literature Eugenol content of Tulsi oil is 21%
- For 21 gm of Eugenol required 100 gm of oil therefore 1gm of Eugenol required 4.7 gm of oil. For 10 mg Eugenolrequired 0.047 gm of Tulsi oil.
- From considering this content prepared serial dilution of Tulsi oil (i.e. 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml) in methanol.
- The absorbance of resulting solutions were measured at 281 nm using UV-visible spectrophotometer (Jasco V-630, Japan) against respective solvent blank.

Table 11: Observation of Minimum Inhibitory Concentration (MIC) of Eugenol (Tulsi Oil)

| S. No | Concentration in µg | Observation | | |
|---|---|---|---|---|
| | | After 24 hrs | After 48 hrs | After 72 hrs |
| 1. | 0.25 | (+3) | (+3) | (+3) |
| 2. | 0.5 | (+3) | (+3) | (+3) |
| 3. | 1 | (+3) | (+3) | (+3) |
| 4. | 2 | (+3) | (+3) | (+3) |
| 5. | 4 | (+3) | (+3) | (+3) |
| 6. | 8 | (0) | (0) | (0) |
| 7. | 16 | (0) | (0) | (0) |
| 8. | 32 | (0) | (0) | (0) |
| 9. | 64 | (0) | (0) | (0) |
| 10. | 128 | (0) | (0) | (0) |
| 13. | (+)ve growth control | (+3) | (+3) | (+3) |

(+3) indicates growth
(0) indicates growth does not observe.

Table 12: Observation of Minimum Fungicidal Concentration (MFC) of Eugenol (Tulsi oil)

| S. No | Concentration in µg | observation | |
|---|---|---|---|
| | | After 24 hrs | After 48 hrs |
| 1. | 8 | (0) | (0) |
| 2. | 16 | (0) | (0) |
| 3. | 32 | (0) | (0) |
| 4. | 64 | (0) | (0) |
| 5. | 128 | (0) | (0) |
| 6. | (+)ve growth control | (+3) | (+3) |

(+3) indicates growth
(0) indicates no growth

- Using calibration curve of Eugenol in methanol calculate the drug content. The absorbance of Tulsi oil is given in Table 10.

*In vitro Antifungal Susceptibility Testing of Tulsi Oil*

*Determination of Minimum Inhibitory Concentration (MIC) of Eugenol (Tulsi Oil)*

From preliminary antifungal screening studies range selected for antifungal testing was 128-0.25 µg/ml (128, 64, 32, 16, 8, 4, 2, 1, 0.5. 0.25 µg/ml). From reported literature Eugenol content of Tulsi oil is 21%. From calibration curve of Eugenol marker sample, Eugenol content of Tulsi oil was found to be 1811.31µg/ml. 1ml of Tulsi oil = 1811.31 µg of Eugenol 7.06 ml Tulsi oil = 12800 µg of Eugenol

*Preparation of Stock Solution*

- To prepare working dilution of selected range that is 128- 0.25 µg/ml. The first stock solution required is 12800 µg of Eugenol.
- For 12800 µg of Eugenol stock solution, 7.06 ml of Tulsi oil is required considering the estimated content of Eugenol from Tulsi oil.
- Initial first stock dilution (1280 µg/ml) was prepared by calculation mentioned in CLSI guidelines using equation.

$C1xV1 = C2xV2$

Where,

$C_1$= concentration of Eugenol in Tulsi oil in stock solution of Eugenol (Tulsi oil) (12800 µg/ml),

$V_1$= volume of DMSO taken (1ml),

$C_2$= first concentration of stock dilution to be taken (1280 µg/ml) and

$V_2$= volume adjustment required for first stock dilution to be taken.

12800 µg/ml x 1 ml = 1280 µg/ml x V2

V2 = (12800 µg/ml x 1 ml)/1280 µg/ml

$V_2$= 10 ml

*Preparation of Stock Dilution*

- First set of ten test tubes was prepared and labeled with concentration range starting from 1280-2.5 µg/ml (i.e., 1280, 640, 320, 160, 80, 40, 20, 10, 5, 2.5). To each test tube, 5 ml nutrient broth was added excluding test tube 1 labeled with 1280 µg/ml.
- Ten milliliter (10 ml) of stock solution containing 1280 µg/ml drug concentration was placed in test tube 1. Serial dilution of test tube 1 was performed up to test tube 10 by withdrawing 5 ml of solution (initial stock dilution) from test tube 1 to test tube 2, from test tube 2 to test tube 3 and so on up to test tube 10.

*Inoculum Preparation*

- A loopful of *C. albicans*(MTCC-227) colonies previously maintained were streaked on Sabourd Dextrose Agar (SDA) plate which was then incubated overnight in an ambient-air incubator at $35^0$ C.
- Three to five isolated colonies of similar colony morphology were passed again to SDA plate. This plate was used for the initial inoculum preparation.
- Using the tip of a sterile applicator stick, five isolated colonies of similar colony morphology at least 1 mm in diameter were picked, and added to 5 ml of sterile 0.85% NaCl.
- Vortexed for 15 to 20 s.
- The suspension was adjusted to 85% transmittance at 540 nm using a spectrophotometer by adding sterile 0.85% NaCl. Resulting suspension became 1 x $10^6$ to 5 x $10^6$ CFU/ml
- One milliliter (1 ml) of above suspension was added to 9 ml of Nutrient Broth. Resulting suspension became 1 x $10^5$ to 5x $10^5$ CFU/ml.
- Tubes of this suspension were held at $2^0$ to $8^0$ C for up to 3 hrs till further use.

*Preparation of Working Dilution (Inoculation and Incubation)*

- Second set of 10 test tubes was prepared and labelled with 128-0.25 µg/ml (i.e. 128, 64, 16, 8, 4, 2, 1, 0.5, 0.25 µg/ml)
- To each test tube 9ml of broth solution and 0.9 ml of inoculum was added.
- From stock dilution test tube containing 2.5 µg/ml, 0.1 ml of drug solution was withdrawn and placed in test tube labelled with 0.25 µg/ml containing 9ml of broth

Table 13: Compatibility Study of All Ingredients

| Ingredient | Ratio | Observation |
|---|---|---|
| Tulsi oil: Tween80 | 1:1 | Clear solution |
| Tulsi oil: Span 80 | 1:1 | Clear solution |
| Tween 80 : Span80 | 1:1 | Clear solution |
| Tulsi Oil : Carbopol 934 | 1:1 | Homogeneous white dispersion |
| Tween80 : Carbopol 934 | 1:1 | Homogeneous white dispersion |
| Span80 : Carbopol 934 | 1:1 | Homogeneous white dispersion |

Table 14: Selection of Gelling Agents

| S. No | Gelling Agents | Observation |
|---|---|---|
| 1. | Gelatin(5%) | Oil separation |
| 2. | Gelatin(7%) | Gel formed with rubbery texture |
| 3. | Gelatin(12%) | Gel formed with rubbery texture |
| 4. | Sodium alginate(1%) | Oil separation |
| 5. | Sodium alginate(5%) | Oil separation |
| 6. | Carbopol 934 | Stable gel formed |

- solution and 0.9 ml of inoculums (Dilution of inoculums from 1 x $10^5$ - to 5 x $10^5$ CFU/ml suspension to 1 x $10^4$ to 5 x $10^4$ CFU/ml, takes place in this step). Continue repeating the process until all ten test tubes receive 0.1 ml of the desired concentration (From 2.5 to 1280 µg/ml).
- Two empty test tubes were taken designated as the positive and negative growth controls, at the far right.

*Growth controls*

- For positive growth control, 0.9 ml of final inoculums was added to 0.1 ml of broth.
- For negative growth control, 1 ml of broth was taken in test tube.

*Solvent Control*

Inoculum (0.01 ml) was placed into test tube containing solvent diluted in broth.

*Reading MICs*

➢ Read MICs at 24 and 48 hrs, and score as follows.

- 0 = optically clear
- 1+ = slightly hazy
- 2+ = prominent reduction in turbidity compared with that of the drug-free growth control
- 3+ = slight reduction in turbidity compared with that of the drug-free growth control
- 4+ = no reduction in turbidity compared with that of the drug-free growth control.

Results mentioned in Table 11 and Fig.6

*Determination of Minimum Fungicidal Concentration (MFC) of Eugenol (Tulsi Oil)*

- Using a micropipette, separate 100-µl samples were removed from the MIC test tube, each higher concentration test tube, and the positive growth control test tube.
- Using the tip of the micropipette, each 100-µl sample was spread over half the surface of an SDA plate. The plates were incubated at $35^0$ C.
- Plates were read when colonies on the growth control plate were visible, usually 24 hrs, and again at 48 hrs.
- Any plate with five colonies or fewer was considered negative.
- The lowest concentration for which the subculture is negative was considered the MFC. Results mentioned in Table 12 and Fig.7.

*Preformulation Study*

*Compatibility Study*

*Compatibility Study Based on Turbidity*

The samples of oil and surfactants with drug were kept in 1:1 ratio to check compatibility of each ingredient with each other and compatibility of each ingredient with drug for one month. Samples were initially clear. The compatibility was checked on the basis of any turbidity occurred. Result mentioned in Table 13

*Construction of Pseudo-Ternary Phase Diagrams*

*Procedure*

The pseudo-ternary phase diagrams were constructed using titration method to determine the gelling region and to detect the possibility of making organogel with different possible compositions of Tulsi oil, tween 80 and span80 respectively and water. The ratios of two surfactant (i.e. span80 and tween80) were chosen as 1:1, 1:2, 1:3, 2:1 and 3:1, and such mixtures were prepared. These mixtures were mixed with the oil phase to give the weight ratios of 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. Water was added drop by drop and stirred using a magnetic stirrer until a homogeneous dispersion or solution was obtained. After each addition, the system was examined for the physical appearance. The end point of the titration was the point where the solution becomes cloudy or turbid or formed gel. The quantity of the aqueous phase required making the mixture gel or turbid was noted. The percentages of the different incorporated pseudo phases were then calculated and the same procedure was followed for the other surfactants ratios[12,13].

*Selection of Ratio of Surfactant for Preparation of Organogel*

All the ratios of surfactant were plotted in a triangle to give dotted region in pseudo ternary phase diagram. (Shows in Fig.8) The pseudo ternary phase diagram giving large region among all ratios was selected for further preparation of organogel.

Table 15: Composition of Organogel

| S. No | Ingredients %w/w | A | B | C | D |
|---|---|---|---|---|---|
| 1. | Tulsi oil | 20 | 20 | 20 | 20 |
| 2. | Surfactant | 80 | 50 | 80 | 50 |
| 2A | Span 80 | 40 | 25 | 40 | 25 |
| 2B | Tween 80 | 40 | 25 | 40 | 25 |
| 3. | Carbopol 940 | 0.5 | 0.5 | 0.5 | 0.5 |
| 4. | Water | 30 | 0 | 0 | 30 |
| 5. | Methyl paraben | 0.03 | 0.03 | 0.03 | 0.03 |
| 6. | Propyl paraben | 0.01 | 0.01 | 0.01 | 0.01 |
| 7. | NaOH (0.1N) | q.s | q.s. | q.s. | q.s. |

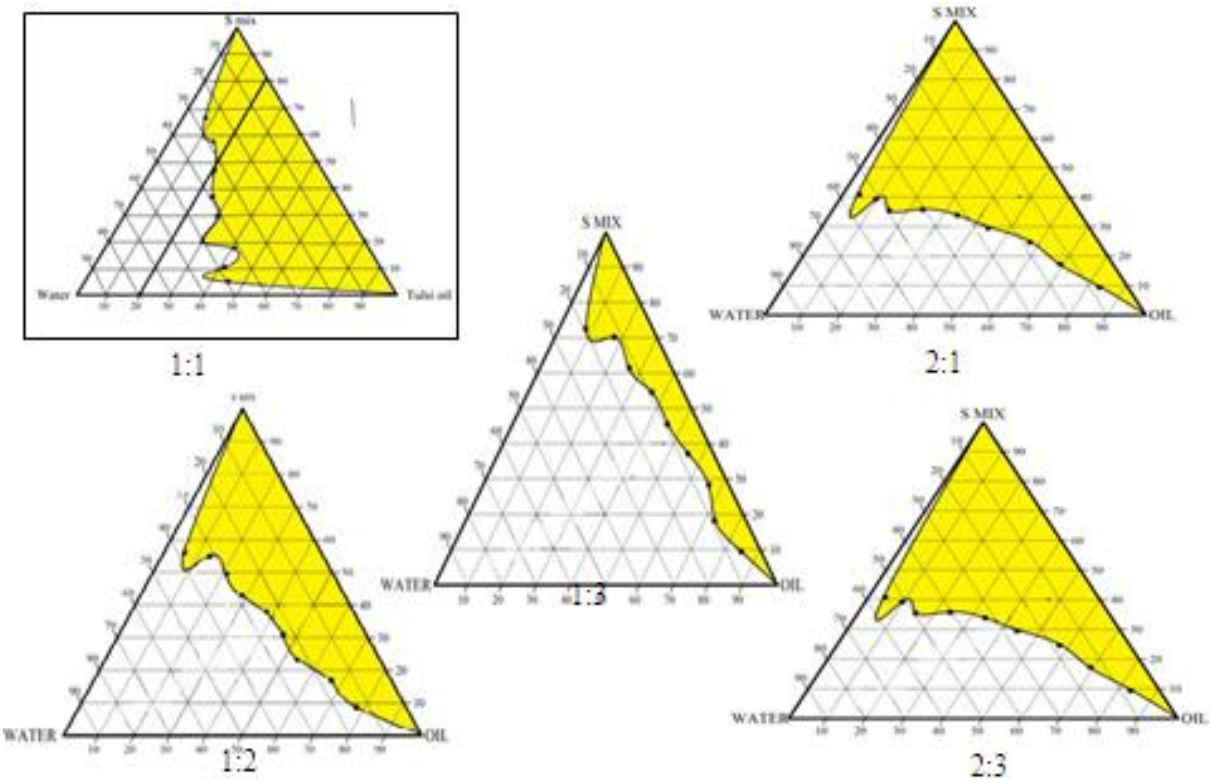


Figure 8: Constructions of Pseudo-Ternary Phase Diagrams

Table 16: pH of different organogel formulations

| Formulation | pH |
|---|---|
| A | 6.5333±0.0577 |
| B | 6.4333±0.0577 |
| C | 6.5667±0.1155 |
| D | 6.7±0.1 |

(n=3)

*Selection of Gelling Agents*

Various gelling agents with different concentration were screened for formation of gel which is mentioned in Table 14.

*PART I: ORGANOGEL*

*Formulation Development of Organogel*

*Factorial design*

A $2^2$ randomized full factorial design (FFD) was constructed where the amounts of Surfactant concentration ($X_1$) (50-80) and water ($X_2$) (0-30) were selected as the independent factors. The two levels and two factors were selected on the basis of the preliminary studies carried out before implementing. The levels of these factors were selected as given in Table 1. The data was analyzed using Design Expert Software 7.0.

*Selection Optimized Batch*

The optimized batch was selected from given solution in Design Expert Software 7.0 on basis of high percentage of drug release.

*Composition of Optimized Batch Organogel*

*Procedure for Preparation of Organogel*

*Evaluation of Organogel*

*Part B: Evaluation of Optimized Organogel Formulation*

Table 17: Viscosity of different organogel formulation

| RMP Formulation batches | Viscosity(cps) A | B | C | D |
|---|---|---|---|---|
| 0.3 | 73430 | 60450 | 70980 | 65780 |
| 0.5 | 70530 | 58450 | 68930 | 64320 |
| 0.6 | 65270 | 53890 | 65290 | 61230 |
| 1 | 60550 | 51000 | 61100 | 56740 |
| 1.5 | 56430 | 48560 | 56730 | 51230 |
| 2 | 51230 | 45720 | 54760 | 49740 |
| 2.5 | 45760 | 41890 | 51430 | 45670 |
| 3 | 41450 | 38560 | 50960 | 41230 |
| 4 | 35640 | 31570 | 45670 | 34590 |
| 5 | 30245 | 25670 | 41360 | 32450 |
| 6 | 29750 | 21790 | 34560 | 26540 |
| 10 | 24570 | 20890 | 29860 | 25430 |

*Characterization of Organogel by Infra-red Spectrophotometer (FTIR)*

The IR spectra of was recorded using fourier transform infra-red spectrophotometer (SHIMADZU, Japan) with diffuse reflectance principle. Sample preparation kept between potassium bromide (KBr) cells, finally placing in the sample holder. The spectrum was scanned over a frequency range 4000 – 400 $cm^{-1}$.

*Physical Appearance*

The prepared Tulsi oil organogel formulations were inspected visually for their colour, appearance and consistency[11,14].

*pH*

pH of all formulations were determined by using pH meter (DIGITAL pH METER MK VI). The pH meter was calibrated before used with standard pH 4 and 7 buffer

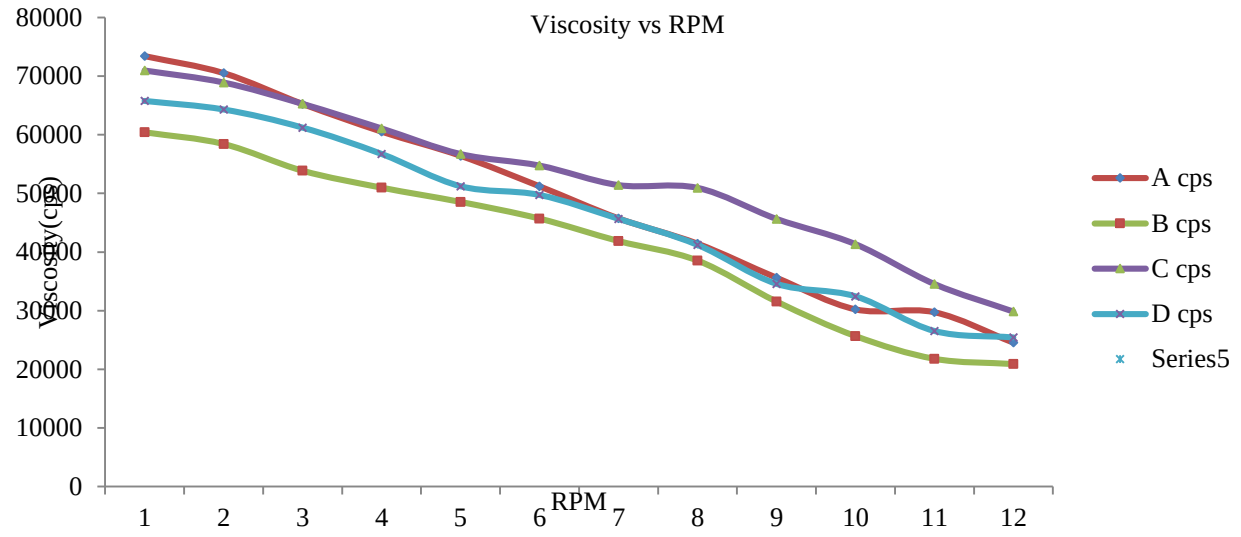


Figure 9: Viscosity of different organogel formulations with respect to rpm

Table 18: Spreadability of all Organogel Formulations

| Formulations | Spreadability (gm.cm/sec) |
|---|---|
| A | 1.9167 |
| B | 2.1623 |
| C | 2.85 |
| D | 2.16 |

Table 19: Drug Content of Different Organogel Formulations

| Formulation | Drug content (%) (Mean±S.D.) |
|---|---|
| A | 95.633±0.550 |
| B | 99.987±3.363 |
| C | 85.233±2.5541 |
| D | 87.003±0.235 |

(n=3)

solutions. Electrode of pH meter dip in beaker containing formulation and pH of the formulation was measured using pH meter in triplicate[11,15].

*Rheological Study*

The viscosity of different organogel formulation was determined at 37$^0$C using a Brook field viscometer. The viscosity was measured by using spindle 64. Results mentioned in Table 22[11,14,16].

*Spreadability*

- One gm of formulation was placed between the glass slides. 250 gm weight was allowed to rest on the upper slide for 1 to 2 minutes to expel the two entrapped air between the slides and to provide a uniform film of the formulation.
- The weight was removed and the top slide was subjected to a pull obtained by attaching 35 gm weight over the pulley.
- The time required for moving slide to travel premarked distance i.e. 10 cm was noted.
- The readings obtained were indications of relative spreadability of different formulations. Results mentioned in given *Part B 4.6.5*

It is calculated by using the formula[11,14]. Where, M = Wt. tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides.

*Drug Content Determination*

Drug concentration in organogel was measured by UV spectrophotometer (Jasco V-630, Japan). Eugenol content in organogel was measured by dissolving known quantity of organogel in solvent (methanol) by sonication. Absorbance was measured after suitable dilution at 281 nm in UV spectrophotometer (Jasco V-630, Japan) and % drug content was calculated[11,14]. Results mentioned in given *Part B section 4.6.6*

*In Vitro diffusion Study*

*In vitro* diffusion was carried out by diffusion cell. A glass cylinder with both ends open, 10 cm height, 3.7 cm outer diameter and 3.1 cm inner diameter was used as diffusion cell.

- An egg membrane (soaked in phosphate buffer 24 hours before use) was fixed to one end of the cylinder with the aid of an adhesive to result as a diffusion cell.
- One gm of organogel was taken in the cell (donor compartment) and cell was immersed in a beaker containing 250 ml of phosphate buffer pH 7.4 as receptor compartment.
- The entire surface of the cell was in contact with the receptor compartment which was agitated using magnetic stirrer and a temperature of 37±1°C was maintained.
- Samples (10 ml) were withdrawn from the receptor compartment at 1hour interval of time over a period 8 hours with same amount replaced to maintain sink condition.
- The sample was analyzed for Eugenol at 281 nm against blank using UV Spectrophotometer.
- Concentration of Eugenol released at various time intervals was calculated with the help of calibration curve with phosphate buffer pH 7.4 and plotted against time. Results mentioned in given Table 23, 24[11,14].

*Bioadhesion Strength*

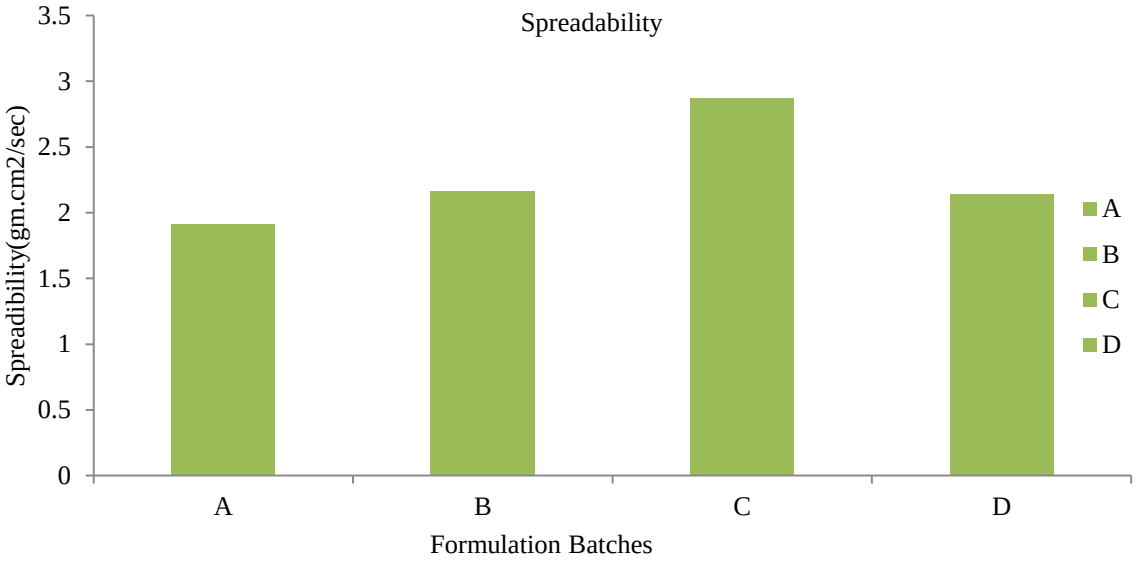


Figure 10: Spreadability of Different Organogel Formulations

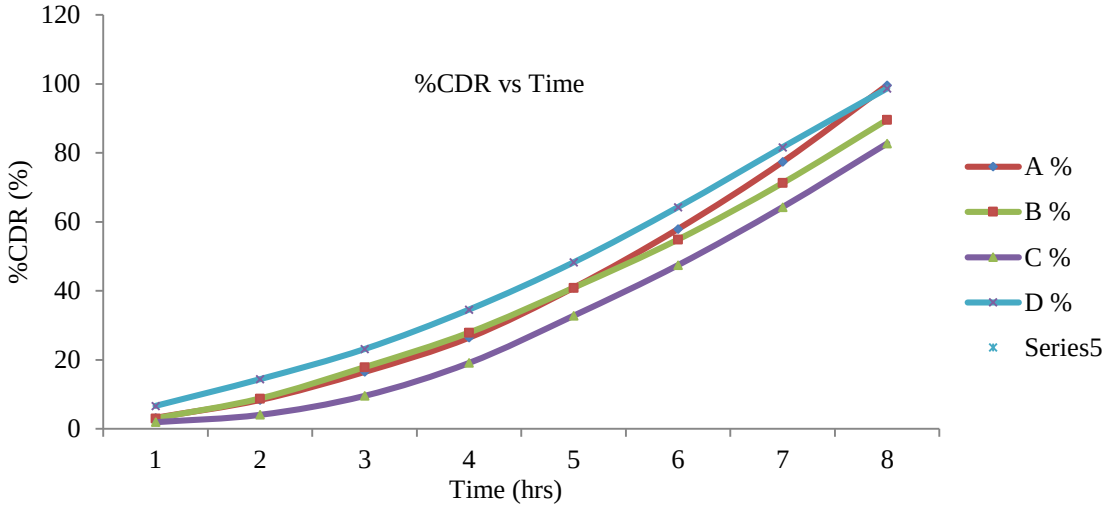


Figure 11: Comparison Pattern of Cumulative Amount of Eugenol Diffused from Organogel Formulations.

Detachment stress is the force required to detach the two surfaces of mucosa when a formulation or gel is placed in between them. The detachment force was measured by using a modified analytical balance an egg membrane (soaked in phosphate buffer (pH 7.4) 24 hours before use) was fixed on a flat surface of object which was moisten with soaked in phosphate buffer (pH 7.4) solution. Another object which is having flat surface sin that surface another membrane was fix which is fixed in position. The height of two objects was adjustable so that membrane surface of both objects can be in intimate contact between membrane and formulation. Then weight was kept rising in the pan until the adjustable object get detached. The weight required to detach the membrane surfaces gave the bioadhesive strength. Results mentioned in Table 25

The bioadhesive strength is calculated by using following

$Bioadhesivestrength = Weightrequired\ (gms)/Area\ (cm2)$[18].

*Skin Irritation Test*[19]

Skin irritation study was performed by using HET- CAM test (Hen's Egg Test – Chorioallantoic Membrane). In order to avoid sacrificing the animal for Skin Irritancy Test, HET CAM test was used. The purpose of this protocol is to describe the components and procedures used to evaluate the potential irritancy of a test substance as measured by its ability to induce toxicity in the chorio-allantoic membrane of a chicken. This test is used because it is intermediate between animal study and human study. Effects are measured by the onset of

- Haemorrhage
- Coagulation and
- Vessel lysis.

These assessments are considered individually and then combined to derive a score, which is used to classify the irritancy level of the test substance.

*Solvents Required*

- 0.9% (w/v) sodium chloride (NaCl) in deionized/distilled water

Table 20: Cumulative Amount of EugenolDiffused (%) from Organogel Formulations Using Diffusion Cell

| Time | % Cumulative drug release (Mean±S.D.) | | | |
|---|---|---|---|---|
| (Hrs.) | A | B | C | D |
| 1. | 3.2±0.445 | 2.99±0.54 | 1.93±0.1153 | 6.62±0.21 |
| 2. | 8.35±0.54 | 8.77±0.39 | 4.06±0.1484 | 14.37±0.21 |
| 3. | 16.48±0.51 | 17.85±0.52 | 9.56±0.3535 | 23.1±0.93 |
| 4. | 26.43±0.68 | 27.88±0.54 | 19.10±0.2695 | 34.55±0.93 |
| 5. | 40.9±1.02 | 40.89±0.42 | 32.78±1.089 | 48.24±0.37 |
| 6. | 57.95±1.43 | 54.89±0.79 | 47.47±1.3739 | 64.27±0.64 |
| 7. | 77.4±1.19 | 71.25±0.79 | 64.28±1.35 | 81.64±0.97 |
| 8. | 99.64±0.25 | 89.62±1.50 | 82.71±1.62 | 98.68±0.77 |

(n=3)

Table 21: Combined Diffusion Kinetic Models for All Formulations

| Formulations | Zero order | First order | Korsmeyer-Peppas | Release Exponent | Higuchi | Hixon-Crowell |
|---|---|---|---|---|---|---|
| A | 0.9573 | 0.9903 | 0.9947 | 1.66 | 0.8863 | 0.9893 |
| B | 0.9743 | 0.9893 | 0.999 | 1.64 | 0.9163 | 0.988 |
| C | 0.9725 | 0.964 | 0.9803 | 1.90 | 0.871 | 0.964 |
| D | 0.9797 | 0.969 | 0.9937 | 1.3 | 0.9243 | 0.9693 |

- 1% (w/v) sodium dodecyl sulfate (SDS) in deionized/distilled water
- 0.1 N sodium hydroxide (NaOH) in deionized/distilled water

*Controls*

*Negative Control*

A 0.9% NaCl negative control should be included in each experiment in order to provide a baseline for the assay endpoints and to ensure that the assay conditions do not inappropriately result in an irritant response.

*Positive Control*

0.1 N NaOH solutions serve as Positive control.

*Preparation of Test Substance*

The tablet was dissolved in a10 ml of 0.9% NaCl solution and used for the study.

*Procedure for HET CAM Test*

*Treatment Groups*

Three eggs per group were required (negative and positive controls, test substance). To the extent possible, eggs from the same hen should be randomized among treatment groups.

*CAM Preparation*

Fresh (not older than 7 days), clean, fertile 50-60 g chicken eggs were selected. Nonviable or defective eggs were discarded by observing under Candle. Excessively misshapen eggs or eggs with cracked or thin shells should not be used. Shaking, unnecessary tilting, knocking, and all other mechanical irritation of the eggs should be avoided when preparing. Eggs were incubated at 38.3 ± 0.2°C and 58 ± 2% relative humidity when incubating in a still-air incubator. Eggs were rotate manually five times per day until day 8. On incubation day 8 eggs were candled and nonviable or defective eggs were discarded. Eggs were returned to the incubator (without hand rotation) with the large end of the eggs upwards for an additional day.

Eggs were removed from the incubator on day 9 for use in the assay. Eggs were again candled discarded if any nonviable or defective eggs present. The air cell of the egg was marked and removed. Care should be taken when removing the eggshell to ensure that the inner membrane is not injured. After preparation of CAM 0.9% NaCl solution was applied on the CAM surface which acts as Negative Control. Positive Control was prepared by applying 0.1 N NaOH solution and incubated at 37 $^0$C for 30 min.Test substance, 0.3 ml was applied on a CAM surface and observed for 30 seconds for any viable reaction.

Table 22: Viscosity of Different Organogel Formulations

| S. No | RPM | Viscosity (cps) |
|---|---|---|
| 1. | 0.3 | 73900 |
| 2. | 0.5 | 70560 |
| 3. | 0.6 | 65780 |
| 4. | 1 | 62340 |
| 5. | 1.5 | 56780 |
| 6. | 2 | 51430 |
| 7. | 2.5 | 43680 |
| 8. | 3 | 35830 |
| 9. | 4 | 25430 |
| 10. | 5 | 18740 |
| 11. | 6 | 12880 |
| 12. | 10 | 75000 |

*Evaluation of Test Results*

The numerical time-dependent scores for lysis, haemorrhage, and coagulation Table 4 is summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21. Result mentioned in Table 26 and skin irritation reaction shows in Fig. 12.

*Anti-fungal Study of Optimized Formulation*

*Media Preparation*

6.5 gm of Sabourd dextrose agar was weighed and transferred in a 250 ml conical flask containing 100ml of distilled water and some amount of heat is applied it completely. Sterilized for 15 min at 121$^0$ C at 15 Ib pressure in autoclave for about 20 min. then cooled at room temperature.

Table 23: Diffusion Kinetic Models for Optimized Formulation

| Formulations | Zero order | First order | Korsmeyer-Peppas | Release Exponent | Higuchi | Hixon-Crowell |
|---|---|---|---|---|---|---|
| Optimized Batch | 0.988 | 0.976 | 0.997 | 1.3 | 0.941 | 0.979 |

Table 24: BioadhesionStrength of Optimized Organogel Formulation

| Formulation | Bioadhesion strength (gram force) |
|---|---|
| Optimized batch | 50 |

Table 25: Observation of Skin Irritation Test

| Effect | Positive control (Score) 0.1 N NaOH | Negative control (Score) 0.9 % Nacl | Test Substance (Score) Tulsi Oil Organogel |
|---|---|---|---|
| Coagulation | 9 | 0 | 0 |

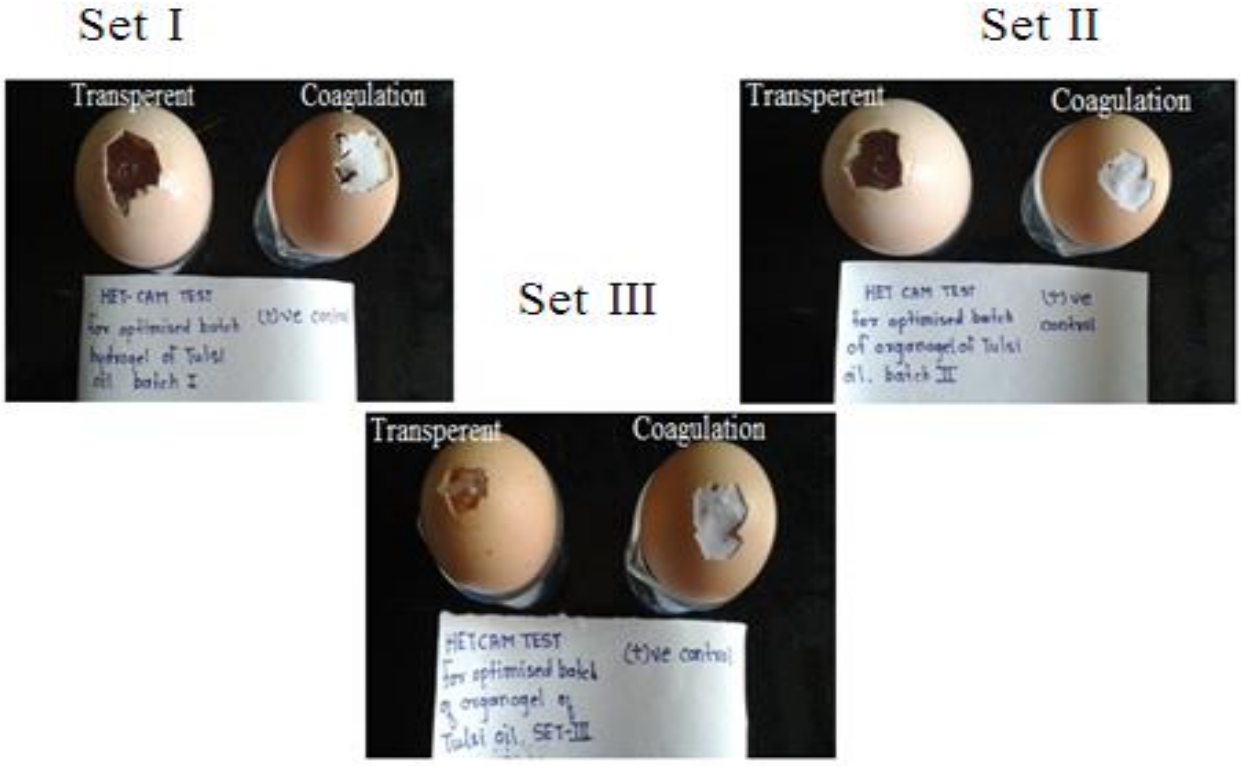


Figure 12: HET CAM Test

*Inoculums Preparation*

- A loopful of *C. albicans*(MTCC-227) colonies previously maintained were streaked on Sabourd Dextrose Agar (SDA) plate which was then incubated overnight in an ambient-air incubator at $35^0$ C.
- Three to five isolated colonies of similar colony morphology were passed again to SDA plate. This plate was used for the initial inoculum preparation.
- Using the tip of a sterile applicator stick, five isolated colonies of similar colony morphology at least 1 mm in diameter were picked, and added to 5 ml of sterile 0.85% NaCl.
- Vortexed for 15 to 20 s.
- The suspension was adjusted to 85% transmittance at 540 nm using a spectrophotometer by adding sterile 0.85% NaCl. Resulting suspension became 1 x $10^6$ to 5 x $10^6$ CFU/ml
- One milliliter (1 ml) of above suspension was added to 9 ml of nutrient broth. Resulting suspension became 1 x $10^5$ to 5x $10^5$ CFU/ml.
- Tubes of this suspension were held at $2^0$ to $8^0$ C for up to 3 hrs till further use.

*Dose selection of Organogel for Antifungal study*

*Ring Worm Infection*

The average area covered by ring worm is 7.85 $cm^2$. The Infection shown in Fig.9

*Minimum Gel Application Requirement*

Area on average of ringworm is 7.85 $cm^2$. The gel preparation is of 20%.20 gmTulsi oil present in 100 gm of organogel

i.e. 36363 µg Eugenol present in 100 gm of organogel therefore, 8 µg Eugenol present in 0.05 gm (50 mg) of organogel

So minimum application of organogel required would be 50 mg. 50 mg organogel required for 7.85 $cm^2$ area of ring worm.

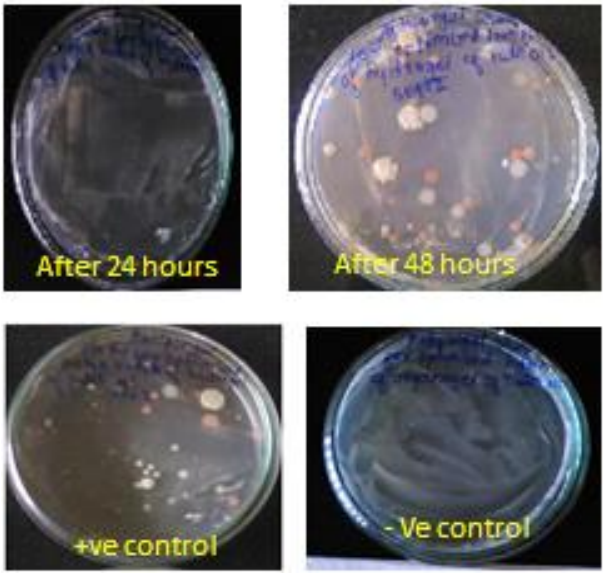


Figure 13: Anti-fungal Study of Hydrogel Formulation

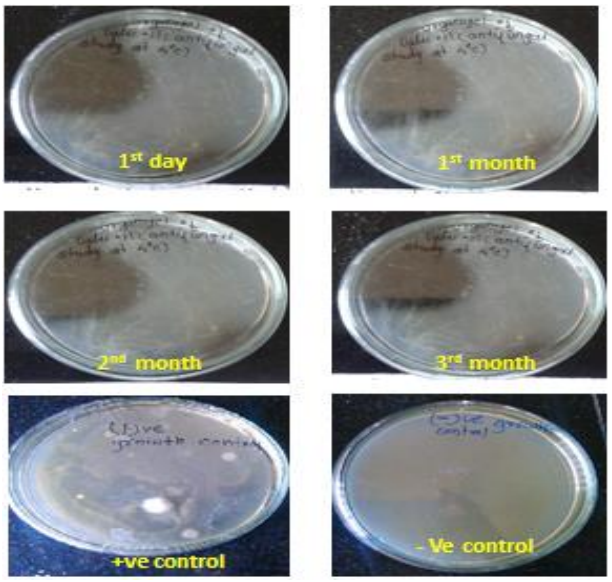


Figure 13: Antifungal study of organogel at $4^0$ C Temperatures

*Area of Petri Plate Selected for Antifungal Study*

From an antifungal susceptibility study MIC obtained in 8µg/ml, so minimum application for an average area of ringworm infection should be 50 mg of organogel (8 µg Eugenol). The area of petriplate taken for antifungal study was 78.57 $cm^2$ which is around ten times more than the calculated area of ringworm infection. The area of petriplate shown in Fig. 3 Accordingly for application of gel formulation on SDA plate during antifungal study the quantity was 50× 10= 500mg of gel formulation on petriplate media surface (having area 78.57 $cm^2$ ).

*Procedure*

The Saboured dextrose agar medium was poured in sterile petri plates and allowed it to cool for some at room temperature until it solidifies. A loopful suspension ($10^5$ CFU/ml) of fungal species was inocuted on petri dish with sterile nichrome wire loop. After that, 0.5 gm of Tulsi oil organogel (without preservative) was spread on petri dish with sterile spreader, and incubated petri plate s upto 72 hrs at $37^0$ c in incubator. Positive and negative growth control were kept for growth observation. The results of antifungal study of formulation kept at $4^0$ C are given in Table 27 and Fig.14. The results of antifungal study of formulation kept at room temperature are given in Table 28 and Fig. 15.

*Part II: HYDROGEL*

*Composition of Hydrogel*

*Procedure for Preparation of Hydrogel*

- 0.5gm of carbopol 934 was weighed.
- 55.88gm of surfactant (span80: tween 80, 1:1) was added in weighed carbopol 934.
- Quantity sufficient water was added making carbopol gel base in above mixture.
- Twenty (20) gm of Tulsi oil was mixed in carbopol base gel.
- 0.1 N NaOHquatity sufficient was added.
- Methyl paraben and propyl paraben in concentration 0.03gm and 0.01gm, respectively was added in final formulation.

*Evaluation of Hydrogel*

Evaluation procedure of Hydrogel is same as Organogel

*Drug Content Determination*

*Anti-fungal Study of hydrogel Formulation*

The results are given in Fig.13

*Stability Studies*[21]

The prepared Tulsi oil organogel and hydrogel formulations were stored away from light in collapsible tube at a) 40°C and 75% RH, b) room temperture and c) $4^0$C for 3 months. After storage the samples were tested for their physical appearance, pH, viscosity, spreadability, drug content, antifungal study and drug release. The results are given in Table 27 and 28.

*Estimation of Shelf Life of Tulsi oil organogel*[22]

It is defined as the time required for the concentration of the reactant to reduce to 90% of its initial concentration. Shelf life is represented as $t_{90}$ and has the units of time/conc. The shelf life equation is given as follows (zero order reaction) $\mathbf{t_{90}} 0.1\, a/k_0$

Where, $t_{90}$= shelf life, a= initial concentration, $k_0$= rate constant

Reaction rate is mathematically expressed in terms of rate constant. Rate constant equation is given as follows (zero order reaction):

$k_0 = (A_0 - A_t) / t$

Where, $k_0$= rate constant, $A_0$= initial concentration, $A_t$= concentration after time‘t’ and

t= time period between initial concentration and concentration after storage for stability study period

*Shelf Life of Organogel at $50^0$C, Room Temperature and $4^0$C*

*Determination of rate constant ($k_0$)*

$A_0$=99.6 % (initial concentration),

$A_t$= 86.64 % (concentration after time‘t’) and t= 28 days (time period between initial concentration and

Table 26: Results of Evaluation when Organogel Kept at $4^0$C Temperature

| Evaluation | On 1st day | On 1thmonth | On 2thmonth | On 3th month |
|---|---|---|---|---|
| pH | 6.67 | 6.5 | 6.4 | 6.28 |
| Viscosity(cps) | 12880 | 11870 | 11870 | 11640 |
| Spreadability (gm.cm/sec) | 2.02 | 2.12 | 2.15 | 2.15 |
| Drug content(%) | 97 | 97 | 91.67 | 80 |
| MIC without Preservative | No growth | No growth | No growth | No growth |
| Drug release(%) | 98.86 | 98.86 | 86.99 | 72.53 |

Table 27: Results of Evaluation when Organogel Kept at Room Temperature

| Evaluation | On 1st day | On 7th day | On 14thday | On 21st day | On 28thday | On 2thmonth | On 3thmonth |
|---|---|---|---|---|---|---|---|
| pH | 6.67 | 6.67 | 6.56 | 6.61 | 6.5 | 6.4 | 6.28 |
| Viscosity(cps) | 12880 | 12350 | 11360 | 11320 | 11360 | 11260 | 10380 |
| Spreadability gm.cm/sec | 2.02 | 2.14 | 2.16 | 2.21 | 2.16 | 2.86 | 3.22 |
| Drug content (%) | 97 | 96.11 | 93.42 | 91.67 | 84.28 | 80 | 75.70 |
| MIC without preservative | No growth | No growth | No growth | No growth | No growth | No growth | No growth |
| Drug release (%) | 98.86 | 91.44 | 89.22 | 86.99 | 88.62 | 72.53 | 60.29 |

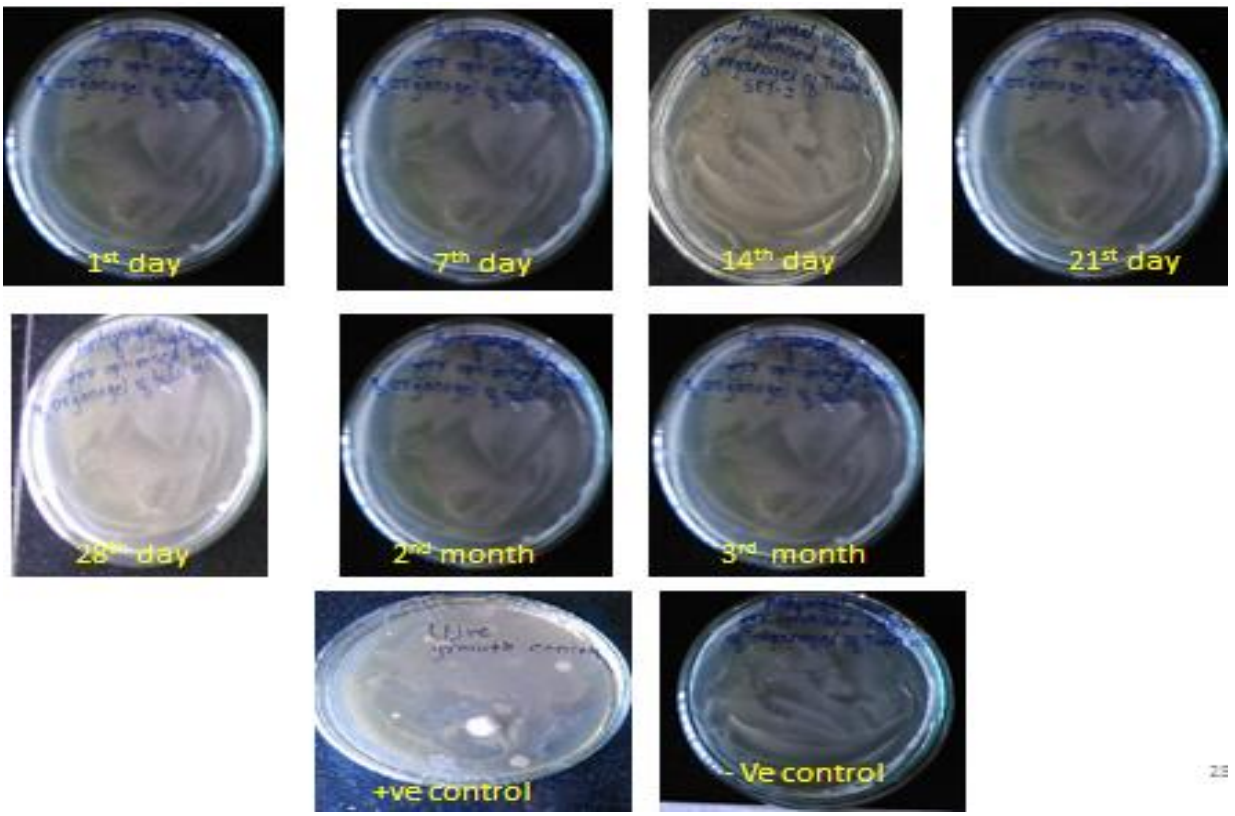


Figure 14: Antifungal study of organogel at room temperature

concentration after storage for stability study period) The result mentioned given in the Table 28.

*Determination of shelf life ($t_{90}$)*

a= 99.6 % (initial concentration),

$k_0$= 0.46/day (rate constant)

The result mentioned given in the Table 28.

**RESULTS AND DISCUSSION**

*Characterization of Tulsi Oil*

*Characterization by FTIR Spectroscopy*

*Characterization of Eugenol by FTIR Spectroscopy*

The IR spectra of Eugenol was recorded and analysed for the functional groups. –OH group is responsisble for antifungal activity.

*Characterization of Tulsi Oil by FTIR Spectroscopy*

The IR spectra of Tulsi oil was recorded and analysed for the functional groups. –OH group is responsible for antifungal activity. The functional group of

Table 28: Rate constant and shelf life Estimation at different temperature.

| Evaluation | Temperature | | |
|---|---|---|---|
| | $4^0$ C | Room temperature | $50^0$ C |
| Rate Constant ($day^{-1}$) | 0.18 | 0.23 | 0.46 |
| Shelf life ( day) | 54 | 42 | 22 |

Eugenolinterpretated in IR spectra of Tulsi oil from that concluded that Eugenol may be present in Tulsi oil.

*Boiling Point*

The boiling point of Tulsi oil was found to be in range of $252^0$C-254°C (Reported 254°C)

*Construction of calibration Curves*

*Determination of maximumwavelenght*

*Determination of maximum wavelenght in Methanol*

Eugenol solution was examined in the range of 400- 200 nm and 0.1 ppm solution of Eugenol in methanol showed absorption maxima at 281 (both solvent).

*Determination of maximum wavelenght in Phosphate Buffer (pH 7.4)*

Eugenol solution was examined in the range of 400- 200 nm and 0.2 ppm solution of Eugenol in phosphate buffer pH 7.4 showed absorption maxima at 281nm.

*Content Estimation of Eugenol from Tulsi Oil*

Using calibration curve of Eugenol in methanol calculated the drug content. Drug content calculalated from straight line equation obtained from calibration curve of Eugenol in methanol.The concentration of Eugenol in Tulsi oil was found to be 1811.3µg/ml

*In vitro Antifungal Susceptibility Testing of Tulsi Oil*

*Determination of Minimum Inhibitory Concentration (MIC) of Eugenol (Tulsi Oil)*

After performing MIC study, concluded that 8µg/mlEugenol (0.044ml Tulsi oil) is minimum inhibitory concentration against *C.albicans* (MTCC-227).

*Determination of Minimum Fungicidal Concentration (MFC) of Eugenol (Tulsi Oil)*

*Preformulation Study*

*Compatibility Study*

*Compatibility Study Based on Turbidity*

After performing compatibility study, it was observed that all ingredients showed clear solution when mixed with each other and doesn't show any turbidity thereafter. Thus, it may be concluded that all ingredients are compatible with each other. Observations of compatibility study of different components are given in Table13.

*Constructions of Pseudo-Ternary Phase Diagrams*

*Selection of Ratio of Surfactant for Preparation of Organogel*

Perecent Composition of Organogel Formulation. The five ratios of span80 and tween80 like 1:1, 1:2, 1:3, 2:1 and 3:1 were prepared and plotted with pseudo ternary phase diagram by using Chemix school software v3.6(trial version). According to region obtained from pseudo ternary phase diagram, the ratio 1:1 is gave best or more region as compare to the other four ratios, so 1:1 ratio of surfactant was selected for the preparation of the organogel. From ternary phase diagram conclude that at constant concentration of Tulsi oil (20%)concentration range of surfactants i.e.span 80:tween80(1:1) and water were found 50-80% and 00-30% respectively.

*Selection of Gelling Agents*

On the basis of stability of carbopol was screened for organogel formation.

*PART A: ORGANOGEL*

*Formulation Development of Organogel*

*1 Formulation and Optimization as Per Factorial Design*

Organogel containing Tulsi oil were prepared as per Table 15

*Evaluation of Organogel*

*Evaluation of Different Organogel Formulation*

*Physical Appearance*

Colour, appearance, consistency of all organogel formulation: white to off white, milky, cream like.

*Determination of pH*

Since topical systems are directly applied on the skin, their pH should be compatible with the skin pH. An acidic or basic pH causes skin irritation or disruption of the skin structure. pH of all formulations were found to be between 6-6.8 which is acceptable for skin preparations.

*Rheological study*

Rheological behavior of the organogel indicated that the systems were shear thinning in nature showing decrease in viscosity at the increasing shear rates. The viscosity data has been summarized in Table 17. All formulations exhibited shear thinning properties.

*Spreadability Study*

The spreadability of different organogel formulations is shown in the figure 28. From the result obtained it was observed that the batch C formulation shows the more spreading coefficient as compared to other formulations. Batch C formulation gives the spreading coefficient 2.85gm. cm/sec which may be due to presence of optimum concentration of gelling agent. Batch B and batch D gives same the spreading coefficient which may be due to equal concentration of surfactants.

*Drug Content Determination*

The drug content of all formulations was found to be in the range of 85 % to 100 %. From reported literature the drug content of Eugenol formulation is more than 80%. [23]. Hence, uniformity of drug content was found to be satisfactory.

In vitro diffusion study

*In vitro* release profiles of eugenol from its various organogel formulations are represented in Fig.38. It was observed that all formulations were found to be swelled at the end of experiment due to penetration of diffusing media into gel matrix which cause breaking of gel matrix, thus release of drug. Additionally three dimentional structure system of organogel contains oil provide enhanced solubility, thus increase in permeation of oil from gel formulation. The higher drug release was observed with formulations A and D. This may be due to presence of maximum amount of water (30%). The formulations A and D showed 99.64% and 98.68% cumulative drug release at the end of 8 hrsTable 20.

*Diffusion Models for Organogel Formulations.*

The dissolution kinetics of formulations was applied to various dissolution models such as Zero order, First order, Higuchi, Korsemayer-peppas and Hixon-crowell. The The order of drug release of formulation A and B were found to be first order, the mechanism of drug release were found to be Korsemayer-peppas as indicated by highest $R^2$ value. Release exponent value 1.66 and 1.64 respectively for Korsemayer-peppas model showed that the mechanism of drug release were case-II transport mechanism indicating pseudo first order type of release profile. It indicates diffusion of drug occur by swelling polymer with simultaneous erosion mechanism. The order of drug release of formulation C and D were found to be zero order, the mechanism of drug release were found to be Korsemayer-peppas as indicated by highest $R^2$ value. Release exponent value 1.3 and 1.9 respectively for Korsemayer-peppas model showed that the mechanism of drug release were case-II transport mechanism. It indicates purely zero order release pattern with diffusion and erosion of drug through swelling polymer[24].

*PART B*

*Evaluation of Optimized Organogel Formulation*

*Physical appearance*

Colour, appearance, consistency of optimized organogel formulation: white to off white, milky, cream like.

*Determination of pH*

The pH of optimized batch was found to be 6.67. Since topical systems are directly applied on the skin, their pH should be compatible with the skin pH. An acidic or basic pH causes skin irritation or disruption of the skin structure. pH of all formulations were found to be between 6-6.8 which is acceptable for skin preparations.

*Rheological study*

*Spreadability Study*

The spreadability of optimized organogel formulation was found to be 2.02 gm.cm/sec.

*Drug Content Determination*

The drug content of a formulation 97% was found to be in the range. Hence uniformity of drug content was found satisfactory and was within acceptance limits.

In vitro diffusion study

*In vitro* release profiles of Eugenol (Tulsi oil) from optimized organogel formulations are represented in Fig.19. It was observed that formulation swelled at the end of experiment due to penetration of diffusing media into gel matrix which cause breaking of gel matrix, thus release of drug.

*Diffusion Kinetic Models for Optimized Formulation*

Release exponent value 1.3 for Korsemayer-peppas model showed that the mechanism of drug release was case-II transport mechanism. It indicates diffusion of drug occur by swelling polymer[24].

*Bioadhesion Strength*

The formulation contained carbopol 934 in the concentration of 0.5% gives bioadhesionstrength 50gram force which shows better adherence to the skin, thus provide sustained release of drug.

*Skin Irritation Test (HET-CAM Test)*

Membrane surface in positive control appear to be coagulation where as the surface remain transparent and clear where formulation are applied indicates evidence for absence of skin irritation at the site of application. Thus, it may be concluded that selected formulation doesn't cause any irritation reaction thus, safe for topical application.

*PART II: HYDROGEL*

*Evaluation of Hydrogel*

*Drug Content Determination*

The drug content of a formulation 97% was found to be in the range. Hence uniformity of drug content was found satisfactory and was within acceptance limits.

*Anti-fungal Study of Hydrogel Formulation*

In antifungal study of hydrogel growth observed after 48 hrs therefore further study discontinued. The observations are shown in Fig. 13

*Stability Study*

(ICH Guidline $Q_1B$, 1996)

*Stability Study of Organogel Formulation*

Short term accelerated stability study was performed at 4 °C for 3 months. After the period of 3 months the organogel formulation was tested for its pH, viscosity, spreadability, drug content, MIC without preservative, drug release, pH, drug content and drug release of formulation was found to be 6.28, 11640, 2.15, 80, no growth 72.53% respectively.

*Stability Study when Organogel Kept at Room Temperature*

*Physical Appearance*

Colour, appearance, consistency of optimized organogel formulation: white to off white, milky, cream like which similar to the day on which it was formulated. Short term accelerated stability study was performed at 4 °C for 3 months. After the period of 3 months the organogel formulation was tested for its pH, viscosity, spreadability, drug content, MIC without preservative, drug release, pH, drug content and drug release of formulation was found to 6.28, 10380cps, 3.22gm. cm/sec, 75.5%, no growth 60.29% respectively.

*Antifungal study: At Room Temperature*

*Esimation of Shelf Life of Organogel Formulation*

## CONCLUSION

Prepared formulation was stable organogel with improved physical stability and prolonged activity. Release exponent value 1.3 for Korsemayer-peppas model showed therefore the mechanism of drug release was case-II transport mechanism. The formulated Tulsi oil organogel showed no irritation after performing skin irritation study using HET-CAM test. After performing anti-fungal study, it was found that the anti-fungal activity of formulated Tulsi oil organogel (optimized batch) is effective against *Candida albicans*. Thus, results of the current study clearly indicate, a promising potential of the anticandidialTulsi oil organogel as an alternative to the conventional dosage form. However, further clinical studies are needed to assess the utility of this system. By considering all above points it was concluded that, the objective of the present research study were achieved successfully.

## CONFLICT OF INTREST

There is no No Conflict of Intrest.

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