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DEVELOPMENT AND CHARACTERIZATION OF MICROSPONGE GEL FOR TOPICAL DELIVERY OF OREGANO OIL

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Keywords:

Microsponges, Oregano essential oil, Psoriasis, GCMS, Antimicrobial activity

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ABSTRACT: Oregano oil is an essential oil possessing anti-inflammatory, antioxidant, antimicrobial, and immune-boosting properties that can be used in the treatment of various skin disorders. However, its practical applications are limited due to its handling issues and poor stability in the presence of air, light, and high temperature. To overcome these problems, this study aims to entrap oregano essential oil (OEO) in ethyl cellulose (EC) microsponges to get decreased dermal toxicity and enhanced stability. The quasi emulsion solvent diffusion method was utilized for fabrication of the OEO microsponges using EC as polymer, dichloromethane (DCM) as a solvent and polyvinyl alcohol (PVA) as stabilizer. The effect of formulation variables like stirring rate, stirring time and quantity of polymer were also examined. The prepared microsponges were evaluated for particle size and production yield. Results revealed that all microformulations were in the micro size range (19.87 mm to 248.13 mm), with a good production yield (72.85%) of M6. Spherical uniform shape with a spongy structure of microsponges was confirmed by Scanning Electron Microscopy (SEM). The optimized batch of OEO microsponges was further formulated into a gel and evaluated for physical appearance, pH, spreadability, viscosity and *in-vitro* release. Antimicrobial activity was also performed using *E. coli*, which indicates that the formulated microgel is safe (on dermal cells) than pure OEO and also confirmed their increased antibacterial effect. Moreover, stability analysis indicated enhanced stability of OEO microsponge gel. Hence, this essential oil became more stable, safe along with better handling due to entrapment in microsponges leading to an efficacious carrier system.

INTRODUCTION: Psoriasis is considered to be a very irritating, chronic, and unpredictable skin disorder, associated with immunological dys-functions of T-cells that affect approximately 2% of the world's population. Although the primary causes for psoriasis are not clearly identified, it is believed as a disorder of keratinocytes [1].

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The treatment of psoriasis varies depending on disease severity and spread. However, topical medications remain the mainstay of psoriasis treatment for most patients.

As seen in the literature, topical corticosteroids, particularly super potent ones, are the most widely prescribed medications for the topical treatment of psoriasis for decades in the world [2]. But serious cutaneous and systemic side effects of the corticosteroids have limited their use. To surmount this issue, there is a strong need to explore safe alternatives, but the development of novel drug moiety is an expensive and time-consuming process [3, 4]. Nature being the chief source of

antimicrobials, might characterize alternative medications, which can help in beating cortico-steroids adverse effects. So, essential oils obtained from natural sources may hold immense potential as the innovative approach to dermatological disorders control in the future [6]. Essential oils are volatile, aromatic liquids and rich sources of bio-actives such as terpenoids, phenols, coumarins, hydrocarbons, and their derivative compounds. They possess wide applications in food/food packaging, cosmetics, and pharmaceuticals, owing to their diverse therapeutic activities. Although the anti-bacterial and antifungal potential of essential oils have long been utilized [6-8], but these have recently been paid more attention as natural antimicrobials for a variety of applications. A number of scientific publications have cited essential oils with clear inhibitory profile effective against a variety of pathogenic fungi and bacteria [9]. Within a huge variety of essential oils, oregano essential oil (OEO) obtained from *Origanum vulgare* L. is well known for its anti-inflammatory, antioxidative, antimicrobial, and immune-boosting properties [10]. These activities are mainly due to the two phenols, carvacrol and thymol (major components of oregano essential oil) and the monoterpene hydrocarbons p-cymene, linalool and Beta-caryo-phyllene [11] which present at lower concentration.

Thymol is a powerful antimicrobial and antifungal agent and has immune-boosting properties that help strengthen a weak immune system, which is a possible cause of psoriasis. This natural plant compound also prevents tissue damage and facilitates speedy healing. Linalool, a derivative of monoterpenes, is known for its powerful anti-bacterial properties that can help fight off bacteria and germs, which aggravate psoriasis. Beta-caryophyllene is good for reducing inflammation in the body, hence especially helpful for psoriasis that is an inflammatory condition [12]. Therefore, by using oil of oregano for psoriasis treatment, you can successfully get your dry itchy, and burning psoriasis patches under control. However, its poor physical properties like light sensitivity, hydrophobicity, and vulnerability to degradation, limits its practical use in pharmaceuticals. For oregano oil, chitosan nanoparticles, Pickering emulsions, nanoemulsions, gel, emulgel like formulations [13], have been reported in the literature. Microencapsulation techniques have got wide acceptance in overcoming the limitations and improving the stability of essential oils during storage. Among microcarrier systems, an advanced delivery system named microsponges has been commercialized for a wide variety of products. Microsponges are polymeric microparticles ranging from 5 to 300 mm in diameter, leads to reduced dose and side effects, stability enhancement and elegance, modification of the drug release rate, and formulation flexibility in comparison to microcapsules and liposomes [14].

Moreover, this delivery system has the advantage of cost-effectiveness and increased payload [15]. Furthermore, these microcarriers have the capability of enhancing the dermatological potential of drug moieties by further incorporating microsponges in suitable topical carriers like lotions, creams, and gels, that's why, this investigation was designed to entrap OEO in microsponges to surmount the limitations of oregano oil to get a versatile and valuable therapeutic product. Numerous methods are available for preparing microsponges, out of which quasi-emulsion solvent evaporation method has been most commonly reported for encapsulation of hydrophobic moieties as it is highly reproducible and simple to perform at lab scale and scale-up.

Among the available variety of polymers, Ethylcellulose (non-swellable hydrophobic polymer) has been explicitly reported for preparing nano and microsponges [16]. Hence, in the present investigation oregano essential oil microsponge gel was fabricated using ethyl cellulose by Quasi-emulsion solvent evaporation method, and successful entrapment of OEO was clarified by FTIR (Fourier transform infrared) spectroscopy, UV spectro-photometry. The shape, morphology, and mean particle size of prepared microsponges were determined by SEM (scanning electron microscopy) and a particle size analyzer. The effect of stirring rate, stirring time, and polymer (EC) on mean particle size and production yield was investigated. Additionally, the *in-vitro* release of OEO from microsponges was also investigated. To increase dermatological benefits and practical applicability, a gel was formulated by incorporating OEO microsponges, as microsponges cannot be used as such on skin. Finally, OEO loaded

microsponge gel was evaluated for *in-vitro* release and anti-microbial assay. Stability analysis was also performed for the selected formulation.

MATERIALS AND METHODS:

Material: Oregano oil was obtained from Katyani exports, New Delhi (India). Carbopol, ethyl-cellulose, and propylparaben were procured from Hi- media, India, and dichloromethane were purchased from Central Drug House, New Delhi. Tween 20 and propylene glycol were procured from Sisco Research Laboratories, Mumbai. Nutrient agar was procured from Hi-media, India. All other solvents and chemicals used were of analytical grade. Distilled water was used during this work.

Methods:

Pre-formulation Studies of Oregano Oil:

Physicochemical Characterization: Physico-chemical properties of oil like solubility, density, boiling point, and color were analyzed and reported.

Authentication of Oregano Essential Oil: The authenticity of oil was verified by Gas chromatography-mass spectrometry (GCMS). The method used for the separation and identification of essential oil constituents. The GC-MS was carried at JNU, Delhi. The essential oil was analyzed by GC-MS on Ultra GCMS QP-2010 Plus gas chromatograph–mass spectrometer equipped with an AB-INOWAX (60m × 0.25mm × 0.25µm). The chemical constituents were obtained by the comparison of retention time of the compound with the one given in library. Percentage of individual components was calculated based on GC peak areas without FID response factor concentration.

Preparation and Optimization of OEO Loaded Microsponges: Oregano essential oil microsponges were prepared by quasi emulsion solvent evaporation method. The internal phase was prepared using ethyl cellulose, oregano oil (100 mg), and dichloromethane (DCM) (10ml). The internal phase was stirred for 15 minutes on the magnetic stirrer. Then the internal phase was poured drop-wise into tween 20 (2 ml) and propylene glycol (3ml) solution in water (100 ml), which is the external phase. After the emulsification process complete, the mixture was continuously stirred for a specified time. After continuous stirring, the microsponges were formed due to the removal of DCM. The microsponges were filtered and dried at 40 °C for 24 h[17].

Numerous parameters are known to affect the fabrication of microsponges. Hence, optimization was attained by varying the polymer concentration (400 mg to 800 mg), stirring rate (800 to 1200 RPM), and stirring time (3 to 6 h) and keeping DCM constant *i.e.,* 10 ml. Then, particle size and production yield were measured for selecting the best formulation.

Physicochemical Characterization of OEO Loaded Microsponges:

Determination of Production Yield: The production yield of prepared microsponges was determined by accurately calculating the initial weight of the raw materials and the last weight of the microsponges [18].

Production yield = Production mass of microsponge × 100 / Theoretical mass (Drug + Polymer)

Determination of Particle Size: Determination of particle size was done by Microtrac (model no. S3500, total solutions in particle characterization) equipped with a Hydro dispersing unit. Particle sizing experiments were carried out by means of laser light diffractometry.

Fourier Transforms Infrared Spectroscopy. In order to observe any interaction, the FTIR spectra of OEO loaded microsponges and OEO alone was subjected to infrared analysis. The observations were recorded with Thermoscientific™ nicolet™ FTIR using the KBr disk after scanning the samples from 400 cm^{-1} to 4000 cm^{-1}.

Scanning Electron Microscopy: The morphology and surface characteristics of the microsponges were analyzed using Scanning Electron Micro-scope. The samples were coated with gold-palladium alloy under vacuum. Coated samples were then examined under SEM; JEOL-JSM, 6100, Japan under vacuum at room temperature. The dried samples were placed on NEM TAPE adhesive paper and photographed.

Determination of Percentage Entrapment Efficiency: A sample of dried microsponges equivalent to 10 mg was taken into mortar pestle,

and a little amount of phosphate buffer of pH 7.4 was added and allowed to stand for 24 h. Then content was transferred into 100 ml volumetric flask, and the volume was made up to 100 ml with phosphate buffer of pH 7.4. The solution was filtered through Whatman filter paper (No. 41). From the resulting solution, 1 ml was drawn and poured into 10 ml volumetric flask, and then the volume was made up to 10 ml with phosphate buffer of pH 7.4. The sample was analyzed next to blank by UV spectrophotometer at 273 nm to determine the drug content. The estimation of drug content and encapsulation efficiency was done using the following expressions:

$$\text{Actual drug content (\%)} = (M_{act} / M_{ms}) \times 100$$

$$\text{Encapsulation Efficiency} = (M_{act}/M_{the}) \times 100$$

Where M_{act} = Actual oregano oil content in weighed quantity of microsponges

M_{ms} = weighed quantity of microsponges

M_{the} = Theoretical oregano oil content in micro-sponges.

***In-vitro* Drug Release Studies:** The *in-vitro* drug release studies were carried out with the dialysis membrane. The dialysis membrane was soaked overnight in distilled water. The time-dependent release study at 0- 6 h was performed. 5 ml of the medium was removed and replaced with fresh medium every hour and quantified spectrophoto-metrically [19]. The release was quantified as follows:

$$\text{Release (\%)} = (\text{Released oil/Total oil}) \times 100$$

Formulation and Evaluation of Oregano Oil Microsponge Loaded Gel: Gels are semisolid systems consisting of dispersion made up of either small inorganic particles or large organic molecules enclosing and interpenetrated by a liquid. Gels are biodegradable, elegant, non-greasy, easily spread-able, easily removable, emollient, transparent, and cosmetically acceptable. They also have good adherence and a long shelf-life. This all makes gel an advantageous topical dosage form. So, the gel was formulated using an optimized batch of microsponges. Considering the safety issues, Carbopol 934 was used as a gelling agent. The gel was prepared by dispersing Carbopol 934 (2g) in purified water (100 ml) with constant stirring at a moderate speed using a mechanical shaker; then the pH was adjusted to 6.5-7.4 using triethanolamine (TEA). Microsponges (100 mg) was dissolved in ethanol (2 ml) and mixed properly by continuous stirring in another beaker. Then propylparaben (0.03g) was added to the above solution. Finally, the microsponges containing solution was poured dropwise into the gel phase and stirred continuously until an even gel was formed [20].

Evaluation Parameters of Microsponge Loaded Gel:

Physical Examination: The prepared gel formulation was inspected visually for its color, appearance, and consistency [21].

Homogeneity: Prepared gel was evaluated for homogeneity visually after the gel had set in the container. It was tested for the appearance and presence of aggregation.

Grittiness: Prepared gel was evaluated for grittiness by taking a small quantity of gel between the thumb and the index finger; the consistency and grittiness of the gel were observed [21].

pH of Gel: The pH of the formulation was determined using a digital pH meter. 1g of the gel was dissolved in 100 ml of distilled water and stored for two hours. The measurement of the pH of the formulation was done in triplicate. The instrument was calibrated before use with standard buffer solutions at pH 4, 7, and 9 [21].

Viscosity: Viscosity measurements were done on Brookfield viscometer (LV DVE, Brookfield Engineering Corporation, USA) by selecting spindle number 64 and speed 50 RPM. Gel (50 g) was kept in 50 ml beaker, which was set till spindle groove was dipped and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor [22].

Spreadability: The efficacy of topical therapy depends on the drug formulation spreadability in an even layer to administer a standard dose. The spreadability studies were carried out to determine the ability of the gel to spread readily on application to the skin or affected area. Spread-ability test was done by using parallel plate method **Fig. 1,** 1g of the gel was placed between two parallel plates (20 × 20 cm), and weight of 125 gm

was tied on the upper slide for 1 min and increase in diameter of the gel was noted [23]. It is calculated by using the formula given below:

$$S = M \times L / T$$

Where, M = weight tied to upper slide, L = length of glass slides, T = time taken to separate the slides

FIG. 1: SPREADABILITY ASSEMBLY

Drug Content: 1 gm of oregano oil loaded microsponge gel was accurately weighed and dissolved using methanol, volume was made up to the mark in 100 ml volumetric flask with methanol. From this 10 ml was pipetted out and diluted to 100 ml with methanol (60:40) and the final dilution was made using distilled water to get a concentration within Beer's range. The absorbance was measured by UV spectrophotometer at 273 nm against blank gel treated in the same manner as a sample [24].

***In-vitro* Drug Release Studies:** The *in-vitro* drug release studies were carried out with the dialysis membrane. The dialysis membrane was soaked overnight in distilled water. The microsponge loaded gel was loaded into the dialysis bag. This dialysis bag was put into beaker containing phosphate buffer of pH 7.4 (500ml). The beaker was put on magnetic stirrer.

The time-dependent release study at 0- 7 h was performed. 5 ml of the medium was removed and was replaced every hour with fresh medium and quantified spectrophotometrically [25]. The release was quantified as follows:

$$\% \text{ Release} = \text{Release oil} / \text{Total oil} \times 100$$

Antimicrobial Activity by Agar Well Diffusion Method: Nutrient agar medium was dissolved in distilled water and sterilized in an autoclave for 15 min at 121 °C at 10 psi and then cooled at room temperature. Agar medium was poured into the petri dish and allowed to cool at room temperature until it solidifies. Then agar plates are inoculated with standardized inoculums of *E. coli*. Then, a hole with a diameter of 6 mm has punched aseptically with a sterile cork, and a volume (40 µg/ml) of the gel formulation is introduced into the well. The plates were incubated at 37 °C and examined after 48 h for the zone of inhibition, if any, around the well. The diameter of zone of inhibition was measured with the help of a scale [26].

Stability Studies: The prepared microsponge gel was packed in a container (30 g) and subjected to stability studies according to ICH guidelines at 40 ± 2°C/75 ± 5% RH (accelerated) and 30 ± 2°C/45 ± 5% RH (real) for a period of 2 months. Samples were withdrawn at 30-days & 60 days' time intervals and evaluated for physical appearance, pH, odor & drug content [27].

RESULTS AND DISCUSSION:

Preformulation Studies:

Physicochemical Characterization of Oregano Essential Oil: Physicochemical characterization of Oregano essential oil (OEO), which includes organoleptic properties, determination of solubility, density, and the boiling point was done, and the results are summarized in **Table 1**.

TABLE 1: PHYSICOCHEMICAL PROPERTIES OF OREGANO OIL

Properties	Result
Solubility	Soluble in methanol, ethanol and dichloromethane and insoluble in water
Relative Density	0.921
Appearance	Clear, mobile liquid
Colour	Pale yellow
Odour	Characteristic, pungent warm spicy odour
Boiling Point	239 °C

GC-MS Analysis: Authentication of oregano oil was obtained by GC-MS (Ultra GCMS QP-2010 model gas chromatograph-mass spectrometer). About 21 chemical constituents were identified, out of which carvacrol, alpha, and beta-caryophyllene, p-cymene, and thymol were found in the majority. These are monoterpenes and possess anti-oxidant, anti-inflammatory, anti-fungal and immune boosting properties. Reported peaks, retention time and percentage of each constituent are given in **Table 2** and chromatogram of oregano oil is given in **Fig. 2**.

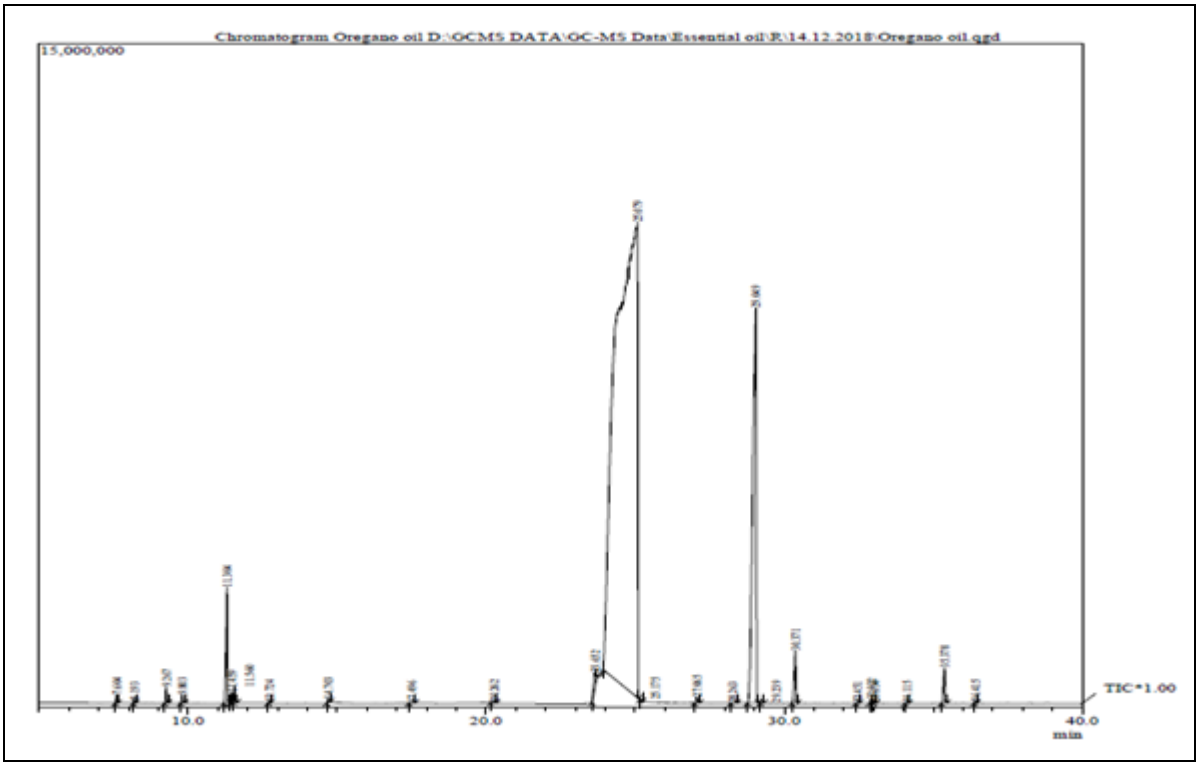

FIG. 2: CHROMATOGRAM OF OREGANO OIL

TABLE 2: CHEMICAL CONSTITUENTS OF OREGANO OIL ANALYSED BY GC/MS MODEL

Peak	R. Time	Area	Area %	Name
1	7.604	503328	0.08	Alpha-pinene
2	8.193	185346	0.03	Camphene
3	9.267	1030758	0.16	Beta-pinene
4	9.803	552687	0.09	Myrcene
5	11.304	9063174	1.44	p-Cymene
6	11.459	675737	0.11	Limonene
7	11.560	1048562	0.17	Cineole
8	12.724	105119	0.02	Gamma-terpinene
9	14.703	348060	0.06	Linalool
10	17.496	58437	0.01	Alpha-santolina alcohol
11	20.262	220170	0.03	Alpha-terpineol
12	23.652	1918062	0.30	Thymol
13	25.079	531202567	84.37	Carvacrol
14	27.065	628642	0.10	Alpha-ylangene
15	29.049	73246022	11.63	Beta-caryophyllene
16	29.239	69991	0.01	Farnesene
17	30.371	4732279	0.75	Humulene
18	32.922	280310	0.04	Cadinene
19	33.034	102685	0.02	Trans-calamenene
20	35.378	3292244	0.52	Caryophyllene oxide
21	36.415	153535	0.02	Humuladienone
		629610867	100.00	

Spectral Analysis:

Development of Calibration Curve of Oregano Oil using UV Spectrophotometer: Calibration curve of oregano oil was obtained by UV spectrophotometer at a wavelength of 273 nm. The values are given in **Table 3,** and the calibration curve of oregano oil is given in **Fig. 3**.

TABLE 3: CALIBRATION CURVE OF OREGANO OIL IN METHANOL: WATER (60:40)

S. no.	Concentration (µg/ml)	Absorbance
1	5	0.241
2	15	0.397
3	25	0.489
4	35	0.656
5	45	0.735
6	55	0.876

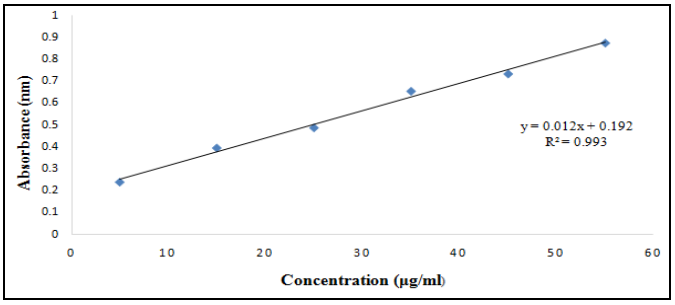

FIG. 3: CALIBRATION CURVE OF THE OREGANO OIL IN METHANOL: WATER (60:40)

Central Composite Design (CCD): CCD was used to study the effect of concentration of polymer (EC), stirring speed, and stirring time on production yield and particle size of various formulations. Production yield and particle size of all the formulations encoded were determined, the results of which are given in **Table 4**. The formulation encoded M6 was found with the highest production yield and minimum particle size.

Optimization of Microsponge Formulation using CCD: Table 4 representing the results of evaluation of all 20 batches of microsponge designed according to central composite design.

TABLE 4: EVALUATED PARAMETERS OF MICROSPONGE FORMULATIONS

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Batch	Concentration of polymer (mg)	Stirring rate (RPM)	Stirring time (hours)	Production yield (%)	Particle size (µm)
M1	600	1000	1.97731	64.28	248.13
M2	600	1000	4.5	71.71	48.21
M3	400	1200	6	58.2	59.12
M4	263.641	1000	4.5	58.29	19.87
M5	400	800	3	61.47	61.32
M6	600	1336.36	4.5	72.85	46.26
M7	400	1200	3	59.1	69.17
M8	800	800	6	62.22	98.23
M9	600	1000	4.5	71.71	48.21
M10	800	1200	3	61.66	115.21
M11	600	663.641	4.5	69.31	76.16
M12	600	1000	4.5	71.71	48.21
M13	800	1200	6	60.55	29.08
M14	600	1000	7.02269	68.14	29.08
M15	800	800	3	63.88	121.12
M16	600	1000	4.5	71.71	48.21
M17	600	1000	4.5	71.71	48.21
M18	936.359	1000	4.5	69.28	261.2
M19	600	1000	4.5	71.71	48.21
M20	400	800	6	60.2	52.31

Characterization of Optimized Batch (M6) of Microsponge Formulation:

Physical Appearance: The prepared microsponges were inspected visually for their color, texture and appearance. Prepared microsponge particles were very fine, fairly white as shown in **Fig. 4**.

FIG. 4: PHYSICAL APPEARANCE OF OREGANO OIL LOADED MICROSPONGES

Particle Size Analysis: Particle size and size distribution of microsponge particles were determined using Microtrac instrument. The average particle size of microsponge formulations should be in the range of 5–300 µm. Visual inspection of all batches done using an optical microscope for particle size and discovered increased particle size with an increase in drug: polymer ratio. It might be since polymer available at higher drug: polymer ratio was in more amount, thereby increasing polymer wall thickness which led to larger size of microsponges. It was also concluded that particle size is decreased upon increasing the RPM and stirring time. The particle size of optimized batch (M4) was found to be diameter 46.26 µm as shown in **Fig. 5**.

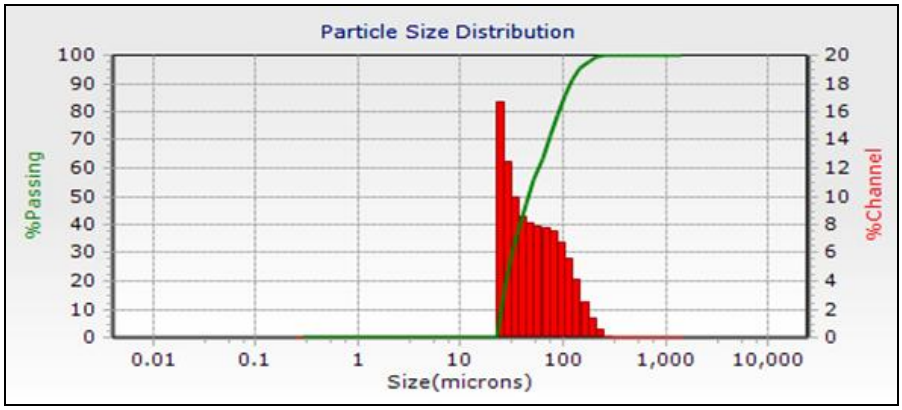

FIG. 5: PARTICLE SIZE DISTRIBUTION OF OPTIMIZED BATCH

Entrapment Efficiency: Drug content in optimized formulation of microsponges was estimated by UV spectrophotometric method. Basically, entrapment of the drug depends on the successful molecular association of the drug with the polymers. The drug entrapment efficiency of the optimized batch of microsponges was found 82.75.

FTIR Characterization (Drug Excipient Compatibility Studies): IR spectra of oregano oil and optimized batch of microsponge *i.e.* M6 were recorded using IR spectrophotometer (Thermo-scientific^{TM} nicolet^{TM} 50) and are shown in **Fig. 6 - 7**. Comparison of peaks is given in **Table 5**. [28]

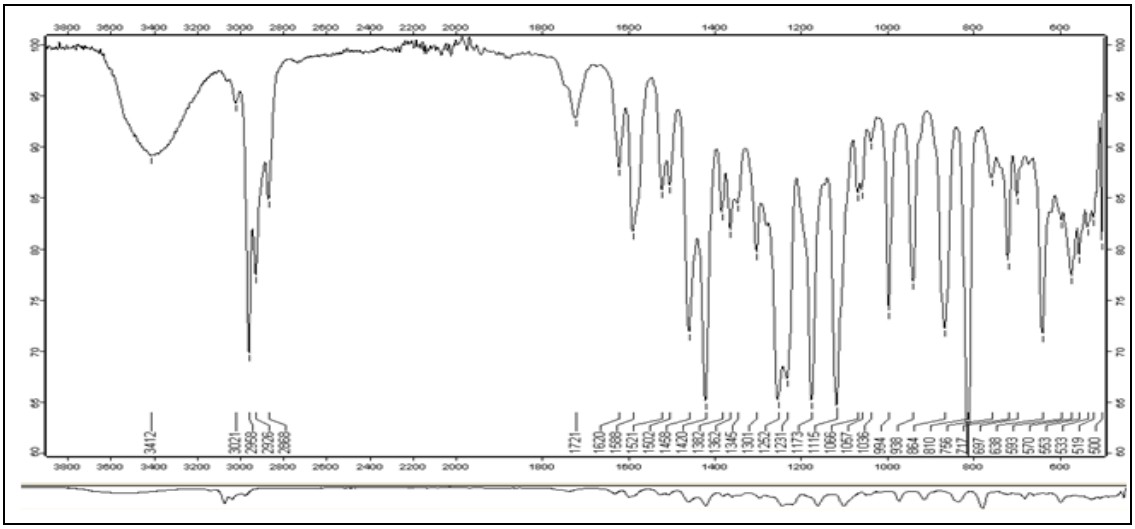

FIG. 6: FTIR SPECTRA OF OREGANO OIL

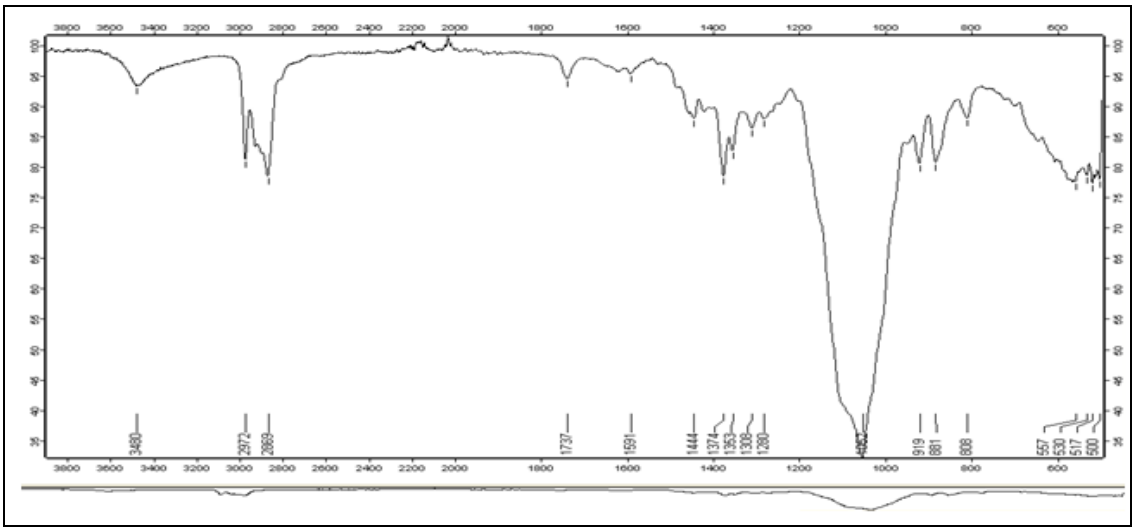

FIG. 7: FTIR SPECTRA OF OPTIMIZED BATCH OF MICROSPONGES

TABLE 5: IR INTERPRETATION OF OIL AND OPTIMIZED BATCH OF MICROSPONGES

Groups	Actual value	Observed value	
		Oregano oil	Optimised batch
-CH stretch	2959	2958	2972
N-H bend	1589	1588	1591
CH_2 bend	1458	1458	1444
C-O-C stretch	1253	1252	1280
C-O-C stretch	1117	1115	1132
C-H bend	937	938	919

As, pure OEO spectra shows sharp characteristic peaks at 2958 (CH stretching), 1588 (N H bending), 1458 (CH_2 bending), 1252 (C O C stretching), 1115 (C O C stretching) and 938 cm^{-1} (C H bending) **Fig. 6**. All the above characteristic peaks appear in the spectra of Oregano oil loaded microsponges **Fig. 7** at closer wavenumber indicating no modification or interaction between the Oregano oil and ethyl cellulose microsponges. The results indicate that Oregano oil might be entrapped into the ethylcellulose microsponges.

Scanning Electron Microscopy: The shape and surface characteristics of microsponges were analyzed using Scanning Electron Microscope (SEM; JEOL-JSM, 6100, Japan) under vacuum at room temperature. It was observed that the microsponges were spherical and uniform with no drug crystals on the surface.

The particle shape was irregular when the stirring speed was low because at low speed, lower energy was produced, and particles stuck together due to no formation of emulsion droplets. As the speed was increased, the shape of microsponges was found to be spherical and uniform. The shape of microsponges was also affected by the amount of emulsifying agent. It was observed that on increasing the concentration of emulsifier, microsponges of large irregular shapes were formed as seen by optical microscopy due to the increased viscosity. The captured SEM image of an optimized batch of microsponges *i.e.*, M6 is shown in **Fig. 8**.

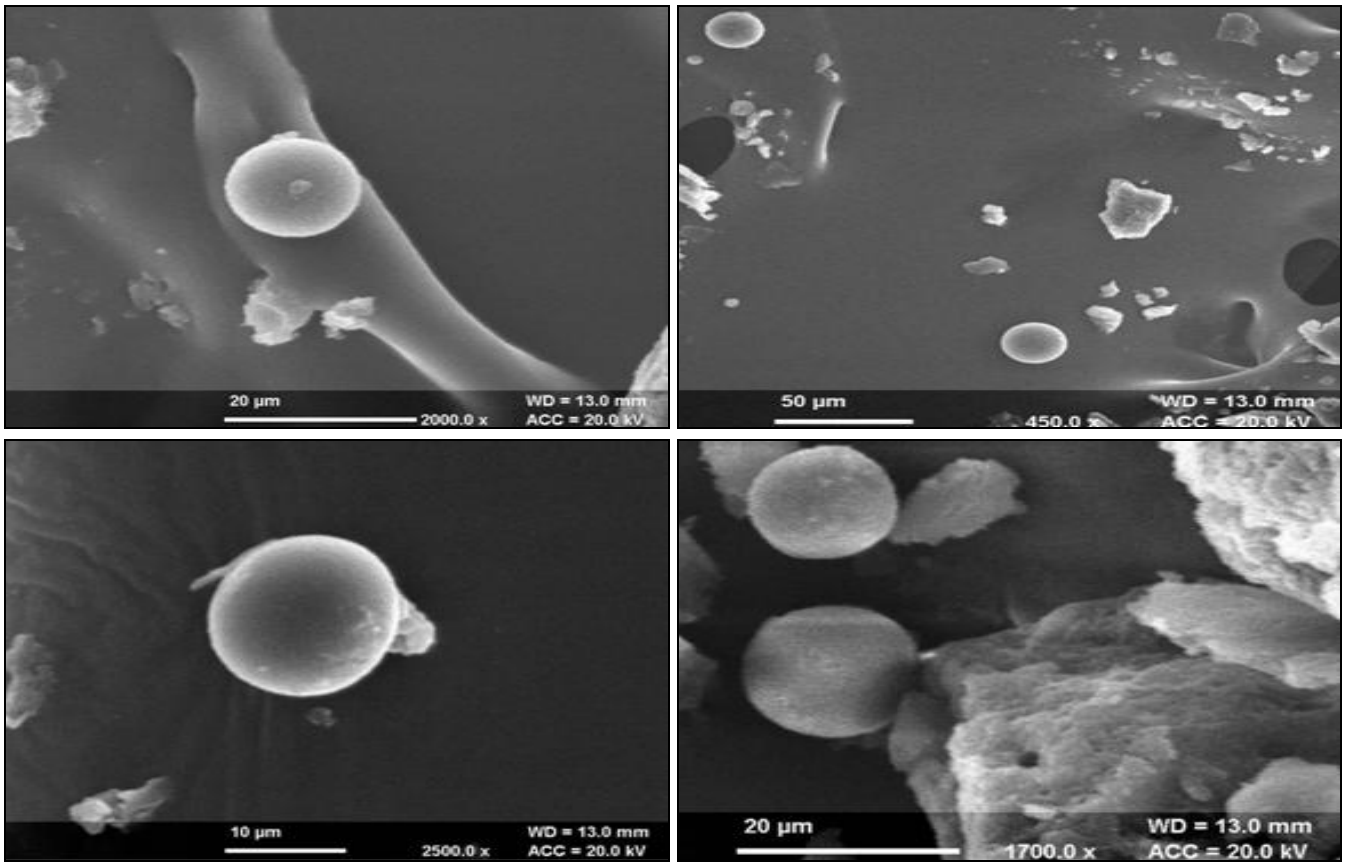

FIG. 8: SEM OF OPTIMIZED BATCH (M6) OF MICROSPONGES

***In-vitro* Drug Release Studies of Microsponges:** The cumulative % drug release of oregano oil loaded microsponges after 6 hrs is summarized in **Table 6**. The graph between cumulative drug release on Y-axis and time on X-axis is shown in **Fig. 9**.

TABLE 6: RESULTS OF *IN-VITRO* DRUG RELEASE STUDIES OF MICROSPONGES

Time (h)	Cumulative % drug release
0	0
1	7.19
2	15.6
3	28.64
4	40.48
5	53.52
6	66

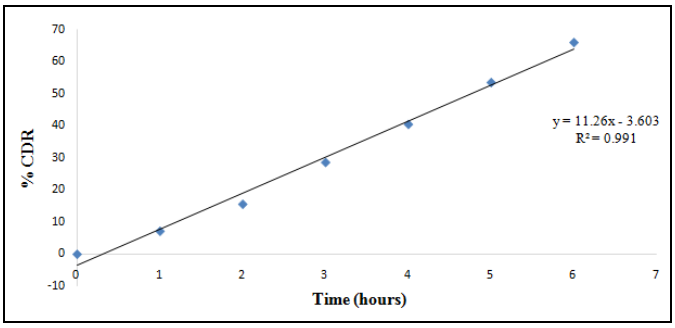

FIG. 9: GRAPH FOR *IN-VITRO* DRUG RELEASE STUDIES OF MICROSPONGES

Evaluation of Microsponge Loaded Gel: The prepared microsponge loaded gel was subjected to evaluation for different parameters, which are as follows:

Physical Appearance: The prepared gel formulation of oregano oil microsponges was inspected visually for their color, texture, and appearance. The prepared formulation was pearl white, viscous preparation with a smooth texture and showed good homogeneity with the absence of any lumps and syneresis **Fig. 10**. Shows the micro-sponge loaded gel.

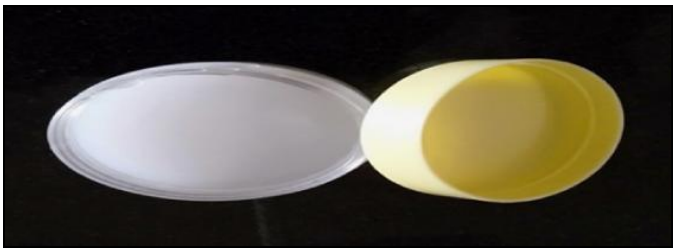

FIG. 10: MICROSPONGE LOADED GEL

Grittiness: The prepared gel was tested for grittiness by taking a small quantity of gel between the fingers. The gel was found free from any particulate matter.

pH Measurement: The pH value of the prepared formulation was found 6.96, which was considered acceptable to avoid the risk of irritation upon application to the skin.

Viscosity Studies: The viscosity study for microsponge formulation was carried out. The viscosity of prepared gel was found to be 42.30 cPs indicating that the viscosity is in the range of required viscosity.

Spreadability Study: The value of spreadability indicated that the gel was easily spreadable by a small amount of shear. The spreadability of microsponge gel was found to be 7.2 cm/sec; indicating that the spreadability of drug-loaded microsponge gel was good.

Drug Content Studies: Drug content studies for microsponge loaded gel were carried out, and the drug content was found to be 81.79%. The drug content of the formulation showed that the drug was uniformly distributed in the gel.

***In-vitro* Drug Release Studies of Gel:** The *in vitro* diffusion studies were carried out for prepared gel formulation using a phosphate buffer solution (pH 7.4). The cumulative % drug release after 7 hrs is summarized in **Table 7**. The graph between % cumulative drug release on Y-axis and the time on X-axis is shown in **Fig. 11**. Drug release kinetic analysis of oregano oil microsponge loaded gel is given in **Table 8,** and the drug release patterns from different models are shown in **Fig. 12** to **15**.

TABLE 7: *IN-VITRO* DRUG RELEASE STUDIES OF LOADED GEL

Time (h)	Cumulative % drug release (% CDR)
0	0
1	6.12
2	14.76
3	26.23
4	34.21
5	46.45
6	55.34
7	66.43

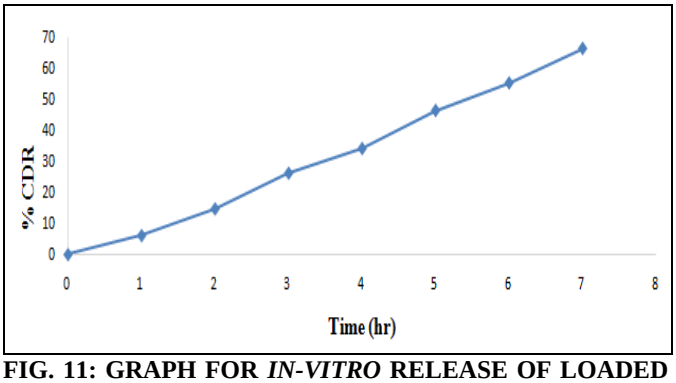

FIG. 11: GRAPH FOR *IN-VITRO* RELEASE OF LOADED GEL

TABLE 8: KINETIC ANALYSIS OF THE OREGANO OIL MICROSPONGE LOADED GEL

S. no.	Time (h)	% Cumulative Drug Released	Square Root of Time (h)	Log of Time (h)	Log of % Cumulative Drug Released	Log of % Cumulative Drug Remaining
1	0	0	0	0	0	2
2	1	6.12	1	0	0.78	1.97
3	2	14.76	1.4	0.3	1.16	1.93
4	3	26.23	1.73	0.48	1.41	1.86
5	4	34.21	2	0.60	1.53	1.81
6	5	46.45	2.23	0.69	1.66	1.72
7	6	55.34	2.44	0.78	1.74	1.64
8	7	66.43	2.64	0.85	1.82	1.52

Zero Order Plot: A curve is plotted between time taken on X-axis and percentage cumulative drug release (% CDR) on Y-axis, as shown in **Fig. 12**.

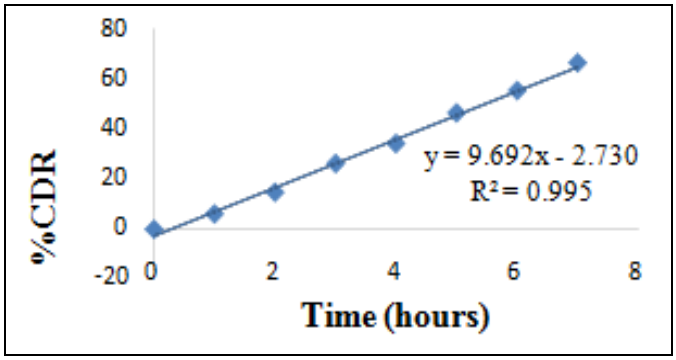

FIG. 12: ZERO ORDER PLOT

First Order Plot: A curve is plotted between time taken on X-axis and log percentage cumulative drug remaining on Y-axis as shown in **Fig. 13**.

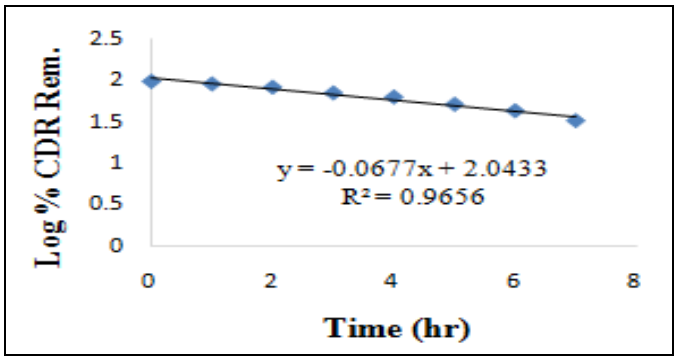

FIG. 13: FIRST ORDER PLOT

Higuchi Plot: A curve is plotted between square root of time on X-axis and percentage cumulative drug release on Y-axis is shown in **Fig. 14**.

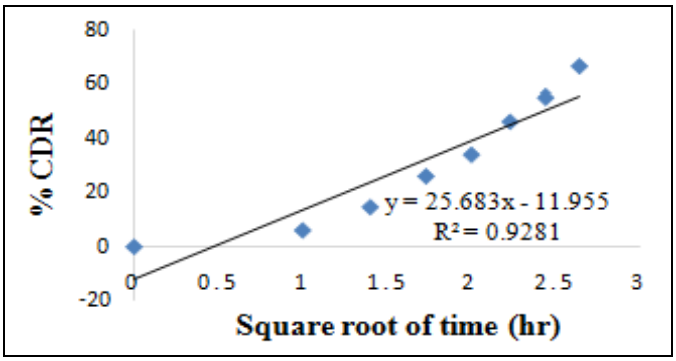

FIG. 14: HIGUCHI PLOT

Korsmeyer -Peppas Plot: A curve is plotted between log time on X-axis and log percentage cumulative rug release on Y-axis is shown in **Fig. 15**.

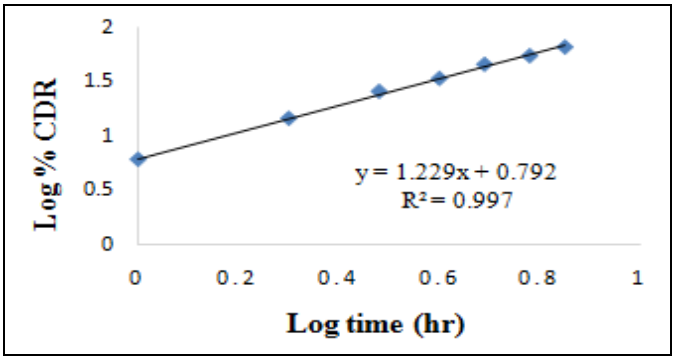

FIG. 15.KORSMEYER-PEPPAS PLOT

Drug release kinetics was studied, and R^2 values of all the plots have been summarized in **Table 9**.

The model that fits the release data was selected based on the correlation coefficient (R^2) value in various models. The model that gave the high 'R^2' value was considered as the best fit of release data.

TABLE 9: R^2 VALUE OF DIFFERENT PLOTS

Formulation	R^2 value of different plots			
Oregano oil loaded microsponge	Zero order plot	First order plot	Higuchi plot	Korsmeyer plot
	0.9956	0.9656	0.9281	0.9979

Korsmeyer peppas model best described the sustained release of optimized M6 formulation. From the result, the best fit model for optimized formulation M6 is the Korsmeyer peppas model.

Comparison of Drug Release Profile of Prepared Microsponge Loaded Gel and Marketed Formulation: *In-vitro* drug release of prepared microsponge gel was compared with a marketed formulation of gel. The cumulative % drug release is summarized in **Table 10**. The graph between % cumulative drug release on Y-axis and the time on X-axis is shown in **Fig. 16**.

TABLE 10: COMPARISON OF DRUG RELEASE OF PREPARED GEL AND MARKETED FORMULATION

S. no.	Time (h)	% CDR of prepared gel	% CDR of marketed formulation
1	0	0	0
2	1	6.12	17.68
3	2	14.76	45.56
4	3	26.23	76.27
5	4	34.21	97.28
6	5	46.45	
7	6	55.34	
8	7	66.43	

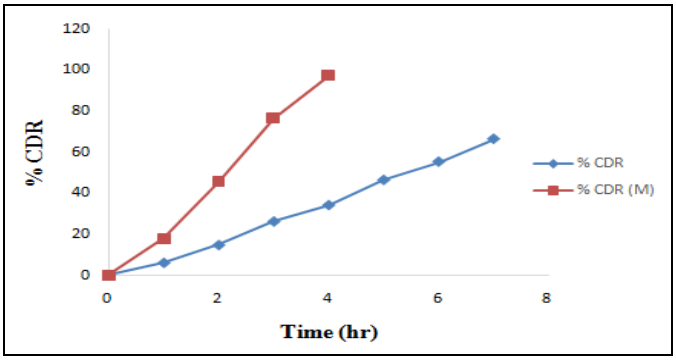

FIG. 16: COMPARATIVE *IN-VITRO* DRUG RELEASE OF MICROSPONGE GEL AND MARKETED FORMULATION

Antimicrobial Activity of Oregano Oil Microsponge Loaded Gel: Antimicrobial activity of oregano oil microsponge loaded gel was performed against *E. coli* using agar well diffusion method. Zone of inhibition (ZOI) of the gel was calculated and was found to be 8.2 mm as shown in **Fig. 17**.

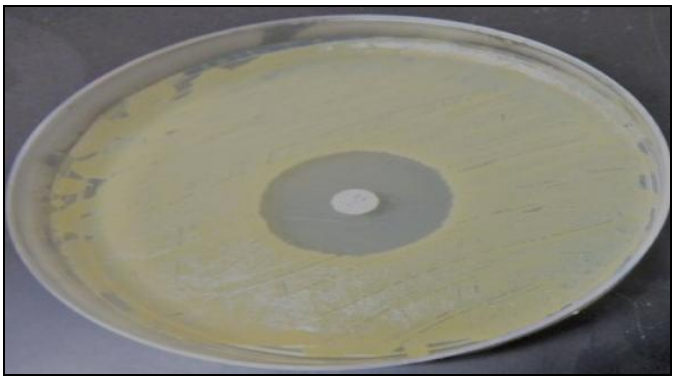

FIG. 17: ZOI OF OREGANO OIL MICROSPONGE LOADED GEL AGAINST *E. COLI*

Stability Studies: The results indicate that there was no evident change in the physical appearance and drug content of formulation after subjecting to stability studies. Optimized formulation M6 was chosen for stability studies. Stability studies on accelerated and real-time were carried out. At a fixed time interval, drug content determination of these formulations showed that there was no significant change in the values when compared to the initial formulations, as shown in **Table 11** and **Table 12**. Thus, we may conclude that the gel does not undergo degradation on storage.

TABLE 11: STABILITY ANALYSIS OF FORMULATED GEL M6 AT 40°C ±2°C/75% RH ± 5% RH FOR 60 DAYS

Parameter	Optimized OEO microsponges loaded gel		
	40°C ±2°C/75% RH ± 5% RH		
	0 Day	30 days	60 days
Colour	White	White	White
Odour	No	No	No
pH measurement	6.96	6.95	6.97
Drug content (%)	81.79	79. 98	77.83

TABLE 12: STABILITY ANALYSIS OF FORMULATED GEL M6 AT 30°C ±2°C/45% RH ± 5% RH FOR 60 DAYS

Parameter	Optimized OEO microsponges loaded gel		
	30°C ±2°C/45% RH ± 5% RH		
	0 Day	30 days	60 days
Colour	White	White	White
Odour	No	No	No
pH measurement	6.96	6.94	6.98
Drug content (%)	81.79	80. 98	79.83

CONCLUSION: Microsponges may prove promising carriers for essential oils but have not been explored for entrapping such bioactive till date. In this study, oregano oil was successfully entrapped in ethyl cellulose microsponges using quasi emulsion solvent diffusion method. The entrapment results in enhanced stability of oregano oil, controlled release, and good payload, along with handling benefits. Surface examination by scanning electron microscopy revealed the porous and spongy structure of microsponges with controlled integrity. Therefore, entrapment of oregano oil in microsponges resulted in a stable, effective delivery system.

Moreover, this system was optimized for psoriasis; by loading prepared microsponges in suitable topical carrier *i.e.*, gel to increase the dermatological potential. Prepared gel was further

evaluated for its physical appearance, pH, spreadability, viscosity, *in-vitro* release studies.

Good *in-vitro* antimicrobial activity on skin pathogen, *E. coli* was also observed. This system also helps to surmount the problem of skin irritation by averting the direct contact of skin and oregano oil.

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