THE DEVELOPMENT, ASSESSMENT, AND *IN-VITRO* INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF AN EMULGEL INCORPORATING *MELISSA OFFICINALIS* OIL

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Abstract

This study aims to develop, characterize, and evaluate an emulgel formulation incorporating *Melissa officinalis* oil, focusing on its *in vitro* antimicrobial potential. Known for its beneficial properties, *Melissa officinalis* (lemon balm) possesses notable antimicrobial activity. The formulation process involved separately creating the emulsion and gel components, utilizing excipients like Carbopol 934, HPMC K100, liquid paraffin, propylene glycol, Tween 80, Span 80, ethanol, and distilled water to optimize stability and consistency. The emulgel underwent extensive evaluations, assessing its physical appearance, pH, homogeneity, spreadability (with EG F3 showing the highest at 6.8 cm), and rheological properties (viscosities between 16,261.66 and 98,651.76 cP). Drug content was quantified at 79.2% to 87.5%, with EG F3 showing the highest incorporation. *In vitro* drug release kinetics indicated a prolonged release profile for EG F3 (52.49% to 55.32% over four hours), suggesting a diffusion-controlled mechanism. Permeation studies revealed cumulative values of 71.98% to 85.92%, with EG F3 achieving the highest rate. The antimicrobial efficacy was confirmed through zones of inhibition against pathogens. Overall, the Melissa officinalis oil emulgel demonstrates promising prolonged release characteristics and significant antimicrobial efficacy, suggesting its potential as an effective therapeutic option for topical applications and laying the groundwork for further research into its benefits.

Keywords: Melissa officinalis oil, Emulgel, Permeation studies and Antimicrobial activity.

Introduction

Herbs and spices have long been incorporated into various foods to enrich their flavor and sensory characteristics. In addition to their culinary use, medicinal herbs play a significant role in traditional and modern medicine, bridging the gap between nutrition and therapeutic benefits [1-4]. The concept of "functional foods," which blend nutritional value with medicinal properties, is gaining popularity. Essential oils derived from medicinal and aromatic plants, such as lemon balm (Melissa officinalis L.), are particularly notable for their potential as natural preservatives in food due to their proven efficacy against a wide range of bacteria [5].

Lemon balm, scientifically known as Melissa officinalis, has a rich historical use as a medicinal herb, appreciated for its distinctive lemon fragrance. Extracted from its leaves and flowering tops, lemon balm oil possesses a complex composition, including prominent components like citronellal and citral [6-11] . This herbaceous perennial, belonging to the mint family (Lamiaceae), has been utilized globally for centuries, primarily in the Mediterranean and other regions. Its pharmacological properties extend to treating conditions ranging from headaches and digestive issues to nervousness and depression, supported by clinical trials demonstrating anxiolytic, antiviral, and antispasmodic effects [12-15].

Material and method

Melissa officinalis oil was purchased from mooty local market, Carbapol 934, HPMC K100, Liquid paraffin and Propylene glycol was obtained from SD fine chemicals, Tween 20, Span 20 and Ethanol was purchased from SD fine chemicals. All other materials and solvents used were of analytical grade.

Pre-formulation study [16-18].

Physical appearance:

The lemon balm oil was visually examined for their texture, color, odor, clarity, and existence of particles.

Preliminary phytochemical test:

Aldehyde:

Fehling's solution is prepared by mixing equal amounts of Fehling's A and Fehling's B solution. The lemon balm oil is taken in a clean test tube. Add Fehling's solution to it and heat the solution gently. If a brick-red precipitate appears, then the presence of aldehyde is confirmed.

Flavonoid:

Test for flavonoids (Shinoda's test) Lemon balm oil was taken and to this, a small amount of magnesium turnings was added; this was followed by 3-5 drops of the concentrated HCl. The intense cherry red color indicated the presence of flavonoids.

Volatile oil:

Lemon balm oil was taken with acidic anhydride and concentrated sulphuric acid, where green color indicates the presence of a volatile oil.

Phenol:

Add a few drops of 1% FeCl3 was added with added lemon balm oil. Yellow color confirms the presence of phenolic compound.

Formulation of emulgel [19-23]:

Preparation of emulsion:

In this method the lemon balm oil is first triturated with gum with little amount of water to form the primary emulsion. The trituration is continued till a characteristics clicking sound is heard and thick white cream is formed. Once the primary emulsion is formed, the remaining quantity of water is slowly added to form final emulsion.

Preparation of gel:

Gels are semisolid dosage form, in that dispersion of small or large molecules in an aqueous liquid vehicle. They produce jelly like consistency by the addition of a gelling agent. Gels are formed by using synthetic polymers such as carbopol 934, HPMC and water.

Incorporation of emulsion to gel:

The emulsion and gel components are carefully combined using suitable equipment, such as a homogenizer, to ensure uniform distribution of the emulsion within the gel matrix. The formulation may be adjusted to achieve the desired consistency, texture, and stability. This can involve fine-tuning the proportions of emulsion and gel components

Table 1: Formulation table

INGREDIENTS	EG F1	EG F2	EG F3	EG F4
Lemon balm oil	2.5	2.5	2.5	2.5
Carbapol 934	1.5	2.5	-	-
HPMC	-	-	1.5	2.5
Liquid paraffin	5	5	5	5
Propylene glycol	2.5	3.5	4.5	5.5
Tween 20	0.6	0.6	0.6	0.6
Span 20	0.9	0.9	0.9	0.9

Ethanol	2.5	2.5	2.5	2.5
Distilled water	q.s	q.s	q.s	q.s

Physical appearance [24-25]:

The prepared gel formulations were visually examined for their texture, color, odor, clarity, consistency and existence of particles.

Measurement of homogeneity and grittiness:

All formulated gels were visually inspected for homogeneity after they were stored in the container. They were examined for their appearance and availability of any aggregates.

Determination of pH:

Accurately weighed 1.0g of various prepared gel and dispersed in 100ml purified water. The pH was measured by using digital pH meter. In order to ensure that the formulation can be used without the harm of skin irritancy, the pH of the preparation has been determined.

Centrifuge test:

Formulation's stability was investigated against gravity by centrifugal device (centrifuge 5430). Each formulation was centrifuged separately inside a tube with 10 cm in length and 1 cm in diameter for 5, 15, 30, and 60 min at 2000 rpm. Finally, each formulation was checked in terms of sedimentation.

Spread ability:

0.5g gel was mounted within a circle of 1 cm diameter pre-marked on a glass plate of 20x20cm and another glass plate was mounted over it. A mass of 100g was placed on the upper glass slide. The change in diameter due to the expansion of gel was reported.

Rheological study:

The viscosity of various emulgel formulations was measured by using Brookfield viscometer.

Drug content:

The prepared emulgel were tested for the drug content uniformity. Accurately weighed quantity for formulation was transferred to a 100ml volumetric flask containing 50 ml of methanol and allowed to stand for 5h with intermittent sonication to ensure complete solubility of drug. The mixture was made up to volume with methanol. The solution was suitably diluted with methanol. The solution was determined by UV.

***In-vitro* drug release and release kinetics [26]:**

A glass cup with a cross-sectional area of 7.5 cm2 was filled with 3 g of the emulgel, covered with a cellulose membrane, sealed with a rubber band, and inverted under the surface of 500

ml of phosphate buffer of pH 5.5 at 37°c ± 0.5°c in a (USP) dissolution tester (pharma test, type II. with a paddle speed of 50 rpm. aliquots were withdrawn at specified time intervals over a 3-hour period and immediately replaced with fresh dissolution medium. The drug content in the withdrawn samples was determined spectrophotometrically using a UV spectrophotometer. The lemon balm oil commercially available in the local market was used for comparison.

A number of kinetic models have been planned to explain the release characteristics of a drug from matrix. The next three equations are usually used, because of their simplicity and applicability.

Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug releasedvs time);

Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and

Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

To study the release kinetics of LVX from the mucoadhesive microspheres the release datawas fitted to these three equations.

Zero order equation:

When a graph of the cumulative percentage of the drug released from the matrix against timeis plotted, zero order release is linear in such a plot, indicating that the release rate is independent of concentration.

$Qt = k0.t$ (1)

Where Qt is the percentage of drug released at time t and k0 is the release rate constant;

First order equation:

$In (100-Qt) = In 100- kI.t$ (2)

Where kI is the release rate constant;

Higuchi's equation:

$Qt = kH.t1/2$ (3)

Where KH is the Higuchi release rate constant

Korsemeyer-Peppas;

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsemeyer's equation.

$Qt/Q\infty = kKP.tn$

Where Qt/ Q∞ is the fraction of drug released at time t, kKPa constant compromising the structuraland geometric characteristics of the device and n is the release exponent. The slopeof the linear curve gives the 'n' value. Peppas stated that the above equation could adequatelydescribe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n

= 1, the release rate is independent of time (typical zero order release / case II transport); n

= 0.5 for Fickian release (diffusion/ case I transport); and when $0.5 < n < 1$, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when $n > 1.0$ super case II transport is apparent. 'n' is the slope value of log Mt/M∞ versus log time curve.

***In-vitro* permeation study [27-32]:**

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

Thermal changes test:

In this test, products were placed at refrigerator (2°C–8°C), room temperature (25°C), and oven (45°C–50°C) then the apparent quality of products was evaluated after 24 h,48h, 1 week

***In-vitro* anti-microbial activity:**

Microbial strains:

The essential oil and the optimized formulation were individually tested against different microorganisms including 2 Gram positive bacteria (Bacillus subtilis ATCC6633, Staphylococcus aureus CIP 7625) and 2 Gram-negative bacteria (klebsiella pneumoniae CIP 8291, Escherichia coli ATCC10536). The bacterial strains were cultured on Mueller-Hinton agar for 48h at 37 °C before use. All microorganisms were regenerated twice before use in the manipulations.

Paper disk diffusion assay:

Paper disk-diffusion method was employed for the determination of antimicrobial activity of the lemon balm oil. Microbial suspensions were prepared in sterile 0.9 % saline and adjusted as inoculum to a final concentration. A volume of 20 mL of Mueller-Hinton agar and Sabouraud, respectively, for bacterial strains was inoculated with 20 µL of microbial

suspension and then poured into a Petri dish. The plates were left at room temperature for 30 min to allow the culture media to solidify. Each paper disk of 6 mm diameter was impregnated with 0.5, 1, 1.5, 2.5 µg respectively, by Emulgel formulation (in methanol) and then applied manually on the surface of the agar plates inoculated with microorganisms. Lemon balm oil (25 µg/disk) were used as positive reference standards to determine the sensitivity of Gram-positive and Gram-negative bacteria species, respectively. The plates were kept at 4°C for 2 h to allow diffusion, and then incubated for 24 h at 37°C for bacteria. The antimicrobial activity was determined by measuring with a zone reader, for the diameters of inhibition zones, including disk diameter.

Determination of minimum inhibitory concentration:

The minimal inhibitory concentration (MIC) test is performed using Mueller Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory growth of most bacterial pathogens. The inoculum is prepared using a suitable nutrient broth.

Preparation of antimicrobial agar plates:

Label each empty sterile plate in order to identify the antimicrobial agent and their concentrations. Example: 0. 25 LB means 0.24 µg/ml of Lemon balm oil. Place the label on the upper portion of the bottom side of the petri dish to ensure that the plate is inserted at the correct point of the basal stand A of the multiple inoculating apparatus. Draw a scheme to locate each bacterial strain in a well on a reference paper that will be used to read the results. Prepare MHA following manufacturer's direction. Keep in a water bath at 48-50°C until use. Pipette 1 ml of appropriate dilutions of the test antimicrobial agent (previously prepared) into the labeled plate (previously prepared). Two replicates must be made for each concentration. Pipette 9 ml of MHA (keep warm at 48- 50°C), add into the plate with appropriate dilution of the test antimicrobial agent and mix thoroughly. Allow the agar to solidify at room temperature and use the plates immediately after the agar surface has dried completely. If necessary, dry the surface of agar in a laminar flow chamber under UV light, but avoid excessive drying.

Inoculation volume:

Inoculate plates with 0.5-2.5 µl of the inoculum if automatic multi-dispenser is used and 10 µl if done manually. Inoculated volume should be uniform for good results.

Inoculation:

Place a completely dried agar plate on the basal stand A of the multiple inoculating apparatus. Insert the plate on the basal stand A of the multiple inoculating apparatus in such a way that the label on the plate is facing the front direction. Place the multiple well tray of the multi-dispenser, which contain 1 ml of inocula, onto the basal stand B to ensure that the marking on the tray is arranged at the front direction. Set the tray to ensure that each inoculator stick will properly dip into the inoculum and carry a uniform volume of each test bacteria. Set the

apparatus to ensure that the surface of each multiple inoculator stick will properly touch the surface of the medium in agar plate. Inoculate the bacterial suspensions onto the surface of the agar plate.

Incubation:

Let the inoculated agar plates remain at room temperature until the moisture in the inoculum spot is absorbed by the agar or until all spots are dry. Incubate the plates in an inverted position at 30°C for 18-24 hours.

Reading of MIC values Lay the agar plates on a non-reflecting dark surface and observe bacterial growth with the naked eye. Place the inoculated plate on or beside the reference paper to identify the spot or position of each tested bacteria. The test should be repeated. Read and record the MIC at the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the naked eye. The MIC is the lowest concentration of antimicrobial agent that completely inhibits colony formation. Disregard a single colony or a faint haze caused by the inoculum. When reporting the MIC values of antimicrobial agents that will prevent the growth of bacterial strains, include important information such as the locality and date the samples were isolated or collected. Evaluate the MIC range of each antimicrobial agent. Report the result as Resistant (R), Intermediate (I) or Susceptible (S).

Result and discussion

Physical appearance:

Table 2: Physical appearance of lemon balm oil

S. No.	Properties	Observation
1	Colour	Pale yellow to light green
2	Odour	Characteristic lemon odor
3	Taste	Mild, Citrusy flavor
4	State	Liquid at room temperature
5	Clarity	Clear
6	Solubility in Aqueous solvents	Slightly soluble
	Solubility in Organic solvents	Miscible in ethanol & acetone
7	Density	0.89 g/ml
8	Volatile nature	Evaporates at room Temperature
9	pH	Typically, acid (4.85±0.2)

The table provided presents a comprehensive overview of the lemon balm oil's organoleptic traits, encompassing its sensory attributes such as taste, smell, and appearance. It appears as a pale yellow to light green liquid with a characteristic lemon odor. It has a mild, citrusy flavor and remains liquid at room temperature. The liquid is clear and slightly soluble in water,

while being miscible in ethanol and acetone. It has a density of 0.89 g/ml and evaporates readily at room temperature. Typically, it exhibits an acidic pH around 4.85 ± 0.2.This detailed analysis serves to confirm the oil's exceptional quality in terms of its sensory appeal and overall characteristics.

Preliminary phytochemical screening test:

Table 3: Preliminary phytochemical screening test

S. No.	Test	Observation	Inference
1	Libermann's test- oil + Acetic anhydride + con. H2SO4	Blue colour was observed.	Presence of volatile oil.
2	Fheling's test- oil+ Fhelings A + Fhelings B Heated in water bath	Brick red colour was observed.	Presence of aldehyde.
3	Shinoda test- Oil+ Mg turnings+con. Hcl	Slightly yellow colour was observed.	Presence of flavonoid.
4	Ferric choride test- Oil+ FeCl3	Yellow colour was observed.	Presence of Phenolic.

The test was conducted to detect phytochemicals in Lemon balm oil. These include phenolics, volatile oils, aldehyde, and flavonoids. The test identified these beneficial compounds within the oil. This finding underscores the rich composition of Lemon balm oil, highlighting its potential health-promoting properties. Further analysis revealed a robust profile of active constituents known for their antioxidant and therapeutic effects. This comprehensive assessment enhances our understanding of Lemon balm oil's phytochemical composition and its potential applications in medicine and nutrition.

Formulation of emulgel:

Emulsion and gel were prepared separately and incorporated successfully. Further evaluation studies were carried out to confirm their activity.

Physical appearance of formulation:

Table 4: Physical appearance of formulation

Formulation Code	Colour	Clarity	Texture	Consistency	Homogeneity	Grittiness	pH	Centrifuge
EGF1	Milky White	Clear	Smooth	Good	Good	No grittiness	4.4	No Phase Seperation
EGF2	Milky White	Clear	Smooth	Good	Good	No grittiness	4.8	No Phase Seperation
EGF3	Milky	Clear	Smooth	Good	Excellent	No	4.9	No Phase

	White					grittiness		Seperation
EGF4	Milky White	Clear	Smooth	Good	Good	No grittiness	5.0	No Phase Seperation

The emulgel containing lemon balm oil was visually assessed to have a smooth texture and creamy white color, demonstrating consistent quality and meeting intended standards effectively. A homogeneity test confirmed uniformity across formulations, with no grittiness observed, ensuring a seamless blend of components. The pH values of EG F1, EG F2, EG F3, and EG F4 ranged between 4.4 and 5.0, closely matching the skin's natural pH of 5.5, which could enhance formulation permeation into the skin for improved efficacy. Components remained uniformly dispersed without phase separation. Spreadability varied among formulations, with EG F3 showing the highest spreadability, particularly notable in HPMC-containing formulations over those with carbopol. Viscosities ranged from 16261.66 to 98651.76, with EG F3 exhibiting the highest viscosity, likely due to its higher drug content facilitated by added gelling agents. These findings collectively suggest that the emulgel formulations are well-suited for topical application, offering smooth application and potentially enhanced skin penetration and efficacy.

Evaluation test for the formulation:

Table 5: Evaluation test for the formulation

Formulation Code	**Spread ability (cm)**	**Viscosity (CP)**	**Percentage drug content**
EGF1	5.5	61261.6	79.2%
EGF2	5.9	16261.66	81.6%
EGF3	6.8	98651.76	87.5%
EGF4	6.2	87342.98	84.1%

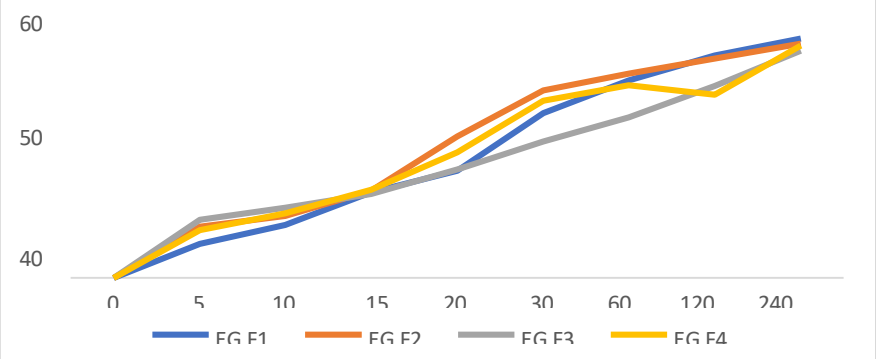
The spreadability measurements for the emulgel formulations were recorded as follows: 5.5 cm for the formulation with a viscosity of 61,261.6 cp and a drug content of 79.2%; 5.9 cm for the formulation with a viscosity of 16,261.66 cp and a drug content of 81.6%; 6.8 cm for the formulation with a viscosity of 98,651.76 cp and a drug content of 87.5%; and 6.2 cm for the formulation with a viscosity of 87,342.98 cp and a drug content of 84.1%. These measurements illustrate varying degrees of spreadability corresponding to the viscosity and drug content of each formulation. Generally, formulations with higher viscosities tend to exhibit lower spreadability, whereas those with higher drug contents may offer enhanced therapeutic efficacy upon application.

***In-vitro* drug release:**

Table 6: *In-vitro* drug release of the formulations

Time (min)	% Cumulative drug release			
	EG F1	**EG F2**	**EG F3**	**EG F4**
0	0	0	0	0
5	7.91	11.84	13.44	11.03
10	12.24	14.30	16.20	14.93
15	19.81	20.23	19.42	20.44
20	24.72	32.70	25.02	29.05
30	38.11	43.38	31.48	40.96
60	45.61	47.18	37.10	44.59
120	51.36	50.17	44.34	49.36
240	55.32	54.15	52.49	53.71

Fig. 7: *In-vitro* drug release of the formulations



The analysis of drug release from formulations EG F1, EG F2, EG F3, and EG F4 revealed varying cumulative release profiles. Specifically, the order of cumulative drug release observed was EG F4 < EG F3 < EG F2 < EG F1, with EG F1 exhibiting the highest cumulative drug release among these formulations. This observed pattern in drug release might be attributed to the concentrations of carbapol and HPMC utilized, suggesting their potential role in retarding the release of the drug from the emulgel.

CUMULATIVE (%) RELEASE Q	0	13.44	16.2	19.42	25.02	31.48	37.1	44.34	52.49
TIME (T)	0	5	10	15	20	30	60	120	240
ROOT (T)	0	2.236	3.162	3.873	4.472	5.477	7.746	10.954	15.492
LOG RELEASE (%)		1.128	1.210	1.288	1.398	1.498	1.569	1.647	1.720
LOG (T)		0.699	1.000	1.176	1.301	1.477	1.778	2.079	2.380
LOG REMAIN (%)	2.000	1.937	1.923	1.906	1.875	1.836	1.799	1.746	1.677
RATE (CUMULATIVE %		2.688	1.620	1.295	1.251	1.049	0.618	0.370	0.219
1/CUM% RELEASE		0.0744	0.0617	0.0515	0.0400	0.0318	0.0270	0.0226	0.0191
PEPPAS log Q/100		-0.872	-0.790	-0.712	-0.602	-0.502	-0.431	-0.353	-0.280
% Drug Remaining	100	86.56	83.8	80.58	74.98	68.52	62.9	55.66	47.51
Q01/3	4.642	4.642	4.642	4.642	4.642	4.642	4.642	4.642	
Qt1/3	4.642	4.424	4.376	4.319	4.217	4.092	3.977	3.818	3.622
Q01/3-Qt1/3	0.000	0.218	0.266	0.322	0.425	0.550	0.665	0.823	

Table 7: *In-vitro* drug release Kinetics of the formulations

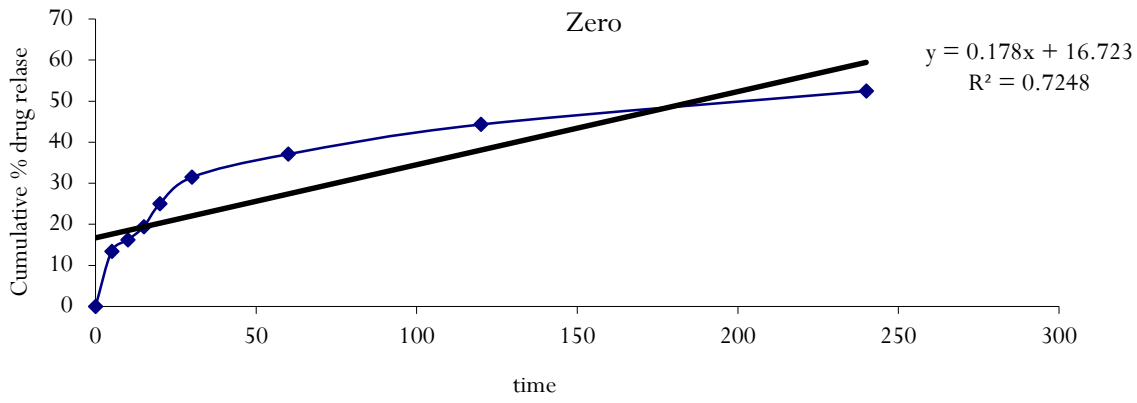


Fig. 8: Zero order release kinetics

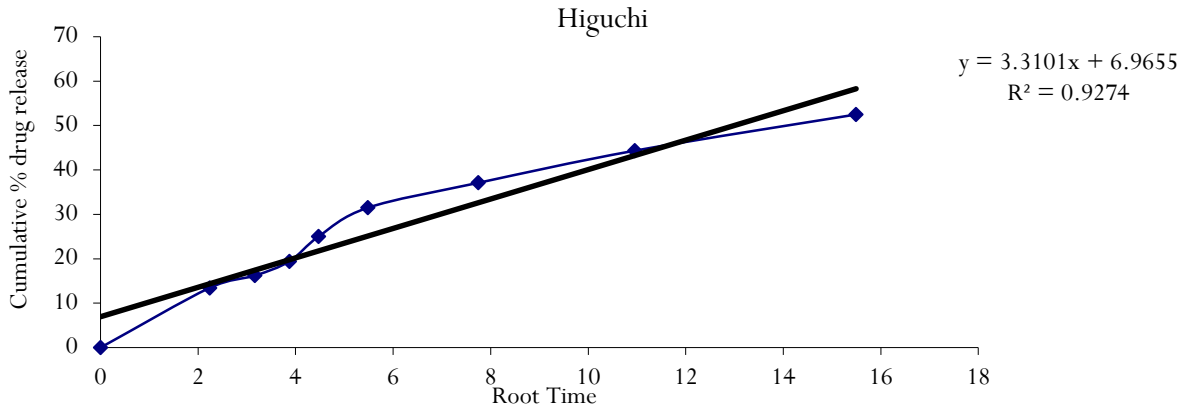


Fig. 9: Higuchi release kinetics

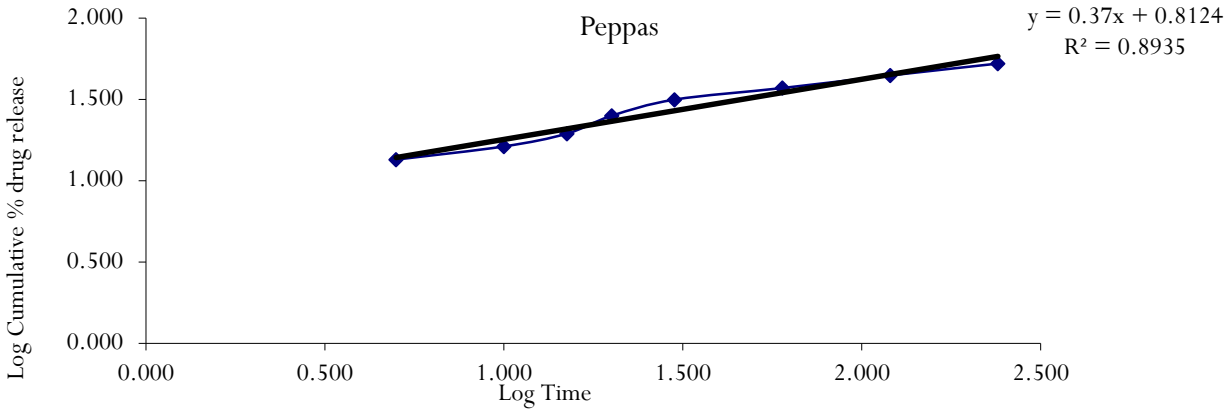


Fig. 10: Peppas release kinetics

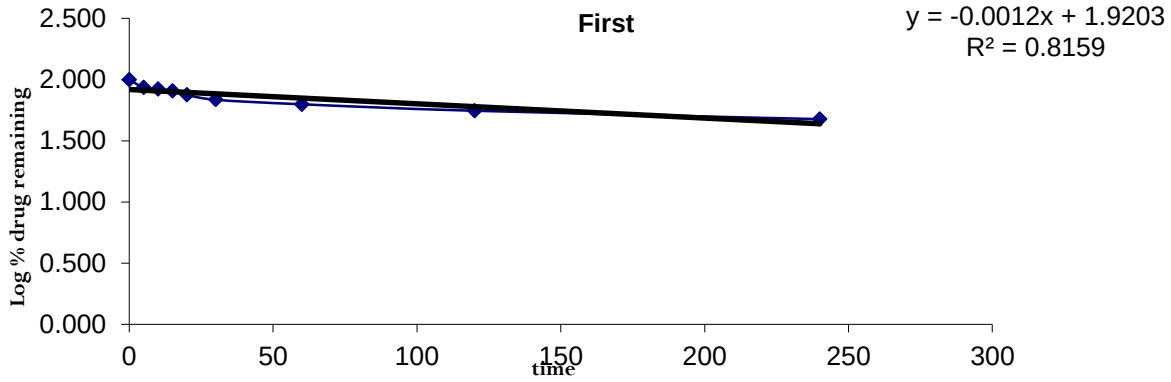


Fig. 11: First order release kinetics

The assessment of drug release mechanism and kinetics involved the application of Higuchi's model, Peppas' model, as well as zero-order and first-order kinetics. Across the formulations, a majority displayed substantial r^2 values ranging from 0.7248 to 0.9274, particularly for Higuchi's model. The notable high regression values obtained from Higuchi's model strongly indicated that the drug release from the emulgels predominantly followed a diffusion-controlled mechanism.

***In-vitro* permeation study:**

Table 8: *In-vitro* permeability study of the formulations

Time (min)	% permeation			
	EG F1	EG F2	EG F3	EG F4
0	0	0	0	0
15	21.45	23.56	27.09	24.87
30	28.98	33.76	35.05	32.01
60	31.89	34.67	36.60	33.98
120	56.98	60.67	69.83	63.75
180	63.87	69.76	73.98	68.54
240	71.98	77.76	85.92	78.56

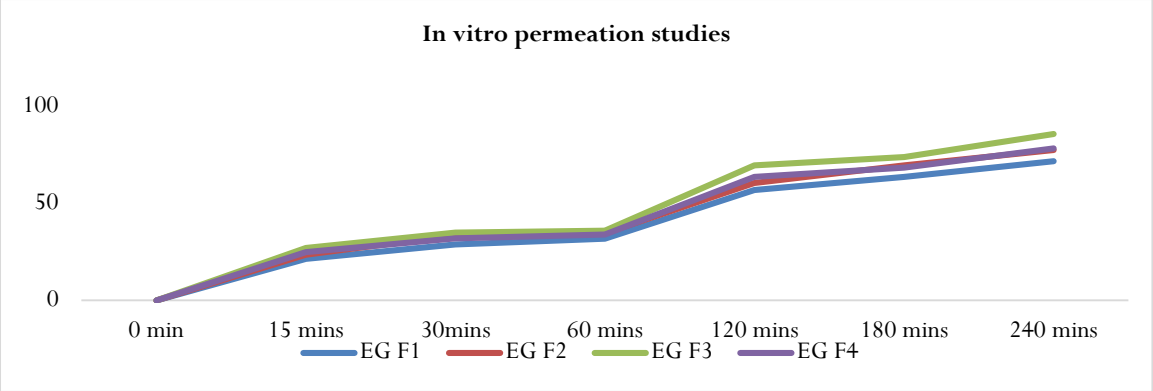


Fig 12: *In-vitro* permeability study of the formulations

The percentage cumulative permeation ranged from 71.98% to 85.92%, with formulation EG F3 achieving the highest penetration value. This superior permeation is attributed to adjustments in the concentration of permeation enhancers within the formulation. The results underscore the formulation's effectiveness in facilitating the absorption of active ingredients through the skin barrier, potentially enhancing its therapeutic efficacy and bioavailability compared to other formulations tested.

***In vitro* anti-microbial activity:**

Table 9: *In-vitro* anti-microbial activity

S.No	Sample	*Bacillus substilis*	*Streptococcus aureus*	*Klebsiella pneumonia*	*Escherichia coli*
1	Melissa officinalis oil	8.4	8.3	8.4	8.5
2	EG F1	7.5	6.9	7.1	7.6
3	EG F2	8.2	7.8	8.1	8.4
4	EG F3	8.8	8.2	8.5	8.9
5	EG F4	7.9	7.2	7.7	8.0

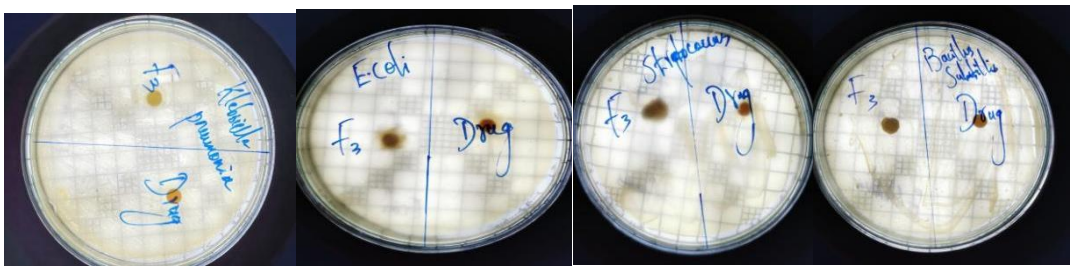


Fig 13: *In-vitro* anti-microbial activity

From the above the results, it was clear about the anti-microbial activity for EG F1, EG F2, EG F3 and EG F4. These results shows that the significant increase of zone of inhibition is in the order of EG F3>EG F2>EG F4>EG F1. This may be due to different concentrations of polymer used in the formulation method. EG F3 showed higher the antimicrobial activity when compared to other formulation, so it was selected as best formulation.

Conclusion

Based on the results, it can be concluded that the formulated Melissa officinalis oil emulgel undergoes prolonged release. The evaluated formulations exhibit potential to reduce the need for frequent drug administration, while also demonstrating antimicrobial activity. These findings suggest that the emulgel could offer sustained therapeutic benefits, enhancing convenience and efficacy in therapeutic applications.

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