

Formulation And Evaluation of Antifungal Emulgel Containing *Citrullus Colocynthis* Fruit Extract

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ABSTRACT:

This study aimed to formulate and evaluate a topical antifungal emulgel containing *Citrullus colocynthis* fruit extract. The ethanolic extract of *Citrullus colocynthis* was prepared using Soxhlet extraction and incorporated into three emulgel formulations (F1, F2 & F3) with varying concentrations of Carbopol 934. The prepared formulations were evaluated for physical appearance, pH, viscosity, spreadability, swelling index, drug content, in-vitro release, antifungal activity against *Candida albicans* and drug – excipients compatibility using FTIR analysis. All formulations exhibited acceptable physical properties with pH suitable for skin application (5.52 – 5.85). Among them, F1 showed optimal viscosity, highest spreadability, maximum drug content (1.237 mg/g), and superior cumulative drug release (58.80% in 6 hours). F1 also demonstrated the greatest antifungal activity (17.25 ± 0.07mm inhibition zone). FTIR studies confirmed the stability of bioactive constituents in the formulation. The results suggest that F1 is a promising herbal emulgel for topical management of superficial fungal infections.

KEYWORDS: *Citrullus colocynthis*, Antifungal Emulgel, Topical Drug Delivery, *Candida albicans*, Evaluation Studies.

I. INTRODUCTION:

1. FUNGAL INFECTIONS:

Fungal infections of the skin are a very common occurrence now a days affecting nearly 40 million people in developing and under developed countries throughout the world. Fungal infections of the body are named according to the site of infection.^[1]

Superficial fungal infections are infections of the human hair, nails, epidermis, and mucosa by pathogenic fungi. The three most popular include

dermatophytosis (tinea or ringworm), pityriasis versicolor (formerly tinea versicolor), and moniliasis (candidiasis) of the skin or genital mucosa.^[2]

Fungi can cause infections of the skin and other, usually deeper, tissues (mycoses). Skin infections due to superficial mycosis are among the most common infectious diseases seen in practice.

Most superficial and systemic fungal infections are treated with either topical/systemic antifungal medications.^[3]

1.2 Topical treatment of fungal infections:

The stratum corneum (the skin's outermost layer) is the best model available for dermal penetration. Other

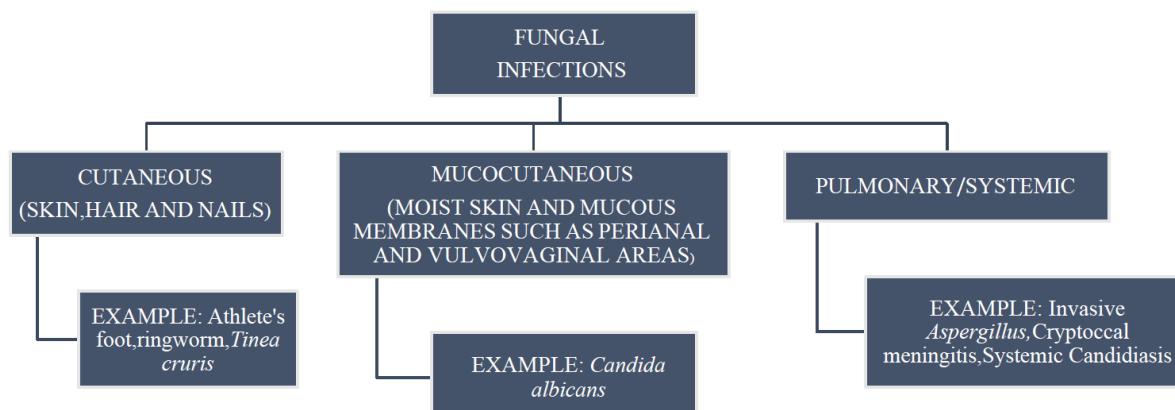


Figure 1: CLASSIFICATION OF FUNGAL INFECTIONS

approaches to improving stratum corneum penetration, such as colloidal drug carriers, micro emulsions, ethosomes, liposomes and niosomes, are being studied.^[4]

For the treatment of fungal infections, there have been a few considerations, including:

- Targeting on the site of disease
- A reduction of the risk of body reactions
- Enhancement of the viability of treatment
- High patient compliance.

Antifungal medications are currently marketed in traditional dose forms, including as lotions, gels, creams, and showers.

When antifungals are applied topically, the therapeutic ingredients must cross the stratum corneum to get to the skin's lower layers, particularly the epidermis. Colloidal systems, vesicular carriers, and nanoparticles are some of the carriers that can improve the delivery of antifungal medicines into the skin. Following dermal application, antifungal medications should reach an effective therapeutic level in viable epidermis.^[5]

2. EMULGEL:

Emulgel is a blend of gels and emulsions; the emulsion can be either water-in-oil or oil-in-water. While water-in-oil systems encapsulate hydrophobic pharmaceuticals, oil-in-water systems directly trap lipophilic medications.

2.1 Emulsion:

Emulsions are biphasic systems in which an immiscible liquid is dissolved into another; emulsifying agents stabilize the system, which becomes unstable as a result. Emulsions are employed as drug delivery vehicles and can be either o/w or w/o. They have high penetration capabilities

and are easily removed from the skin.^[6]

2.2 Gel:

The term "gel" describes the process of increasing the viscosity of liquid preparations without altering other characteristics. Gels can be used as a thickening agent and aid in improving a formulation's homogeneity and consistency; they are smooth, wet, and appear solid. This agent is used to create a gel base, which is then combined with emulsion to create emulgel.^[7]

2.3 Advantages of emulgel:

- It is possible to rapidly introduce hydrophobic medications into the gel basis by using water/oil/water emulsions.
- Emulgels show enhanced steadiness and capability to bear weight.
- A low-cost mechanism with ease of production.
- It stays away from the first pass metabolism.
- Patient compliance.
- Self-medication is made more acceptable and suitable.
- Medicine can be discontinued easily.

2.4 Disadvantages of emulgel:

- People with contact dermatitis might show worsening symptoms due to the medication and its excipients.
- Skin permeability is poor for certain drugs.
- There is a risk of adverse allergic responses.
- Medications with larger particle sizes cannot be delivered.^[8]

2.5 Method of preparation of emulgel:^[8]

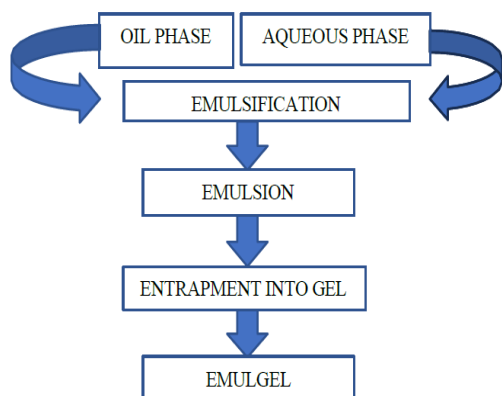


Figure 2: PREPARATION OF EMULGEL

II. CITRULLUS COLOCYNTHIS PLANT:

One source of natural goods for the management and treatment of severe illnesses are medicinal plants. *Citrullus colocynthis*. Schrad., a member of the Cucurbitaceae family, is one such medicinal plant that is mostly used in Ayurvedic literature to treat problems like jaundice, ascites (excess peritoneal fluid), asthma, cough, skin disorders, tumors, wounds, diabetes, and thyroid disorders.^[9]

The term "gel" describes the process of increasing the viscosity of liquid preparations without altering other characteristics. Gels can be used as a thickening agent and aid in improving a formulation's homogeneity and consistency; they are smooth, wet, and appear solid. This agent is used to create a gel base, which is then combined with emulsion to create emulgel.^[7]



Figure 3: CITRULLUS COLOCYNTHIS PLANT

Citrullus colocynthis is a genus of herbaceous plants in the Cucurbitaceae family that

can be either annual or perennial. Distributed throughout the world's desert regions, such as Sudan, Morocco, Jordan, Tunisia, and Pakistan, *C. colocynthis* (L.) Schrad. has both medicinal and nutraceutical uses.^[10]

The National Research Council (2006) states that the mature fruit of *Citrullus colocynthis* has a 90% moisture content. It also has a high protein level (30%), 10% carbohydrate content, 4% ash content, and 3% fiber content.

This medicinal plant has a variety of bioactive substances in addition to these nutritious ones, including alkaloids, flavonoids, glycosides, and essential oils.

Citrullus colocynthis is a fruit that is commonly grown in dry locations and has a variety of medicinal and nutraceutical uses, but it is still not well known and is considered an underutilized fruit crop.^[11]



Figure 4: CITRULLUS COLOCYNTHIS FRUIT

Synonyms: ^[12]

- Bitter Apple
- Colocynth
- Kumattikaai
- Indra Varuni

Traditional uses: ^[13]

Citrullus colocynthis is used extensively across the world to cure a variety of illnesses, such as cancer, mastitis, diabetes, constipation, leprosy, asthma, bronchitis, jaundice, and joint pain.

This plant's medicinal applications have been documented in the indigenous medical systems of Pakistan, India, China, Africa, and Asia. These include the treatment of common colds, coughs, toothaches, wounds, diabetes, and digestive diseases like dysentery, indigestion, gastroenteritis, and colic pain.

Citrullus colocynthis fruit description:^[14]

- About 15 to 30 globular fruits are produced by each bitter apple plant.
- The diameter of fruits is between 7 and 10 cm.
- The fruit has a green skin with yellow stripes covering its exterior part.
- Additionally, the fruits may have a yellow hue. A thin, firm rind is a characteristic of ripe fruits.
- The delicate, white pulp of the fruits is packed with many compressed, ovate seeds.

Medicinal value of Citrullus colocynthis fruit: ^[14]

The fruits treat tumors, ascites, leukoderma, ulcers, asthma, bronchitis, urine discharges, jaundice, enlarged spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia, throat disorders, elephantiasis, and joint pain.

They are also bitter, pungent, cooling, purgative, anthelmintic, antipyretic, and carminative.

III. METHODOLOGY:

Collection of Citrullus colocynthis fruits:

The *Citrullus colocynthis* fruits were collected from the banks of Vellaru River, in and around Ogalur village of Perambalur district.

The collected fruits along with other aerial plant parts was attached to an herbarium paper after sterilizing and drying for one week. The prepared herbarium of the plant was then authenticated by Botanist Dr. Soosairaj, Associate professor, Department of Botany, St. Joseph's College, Tiruchirappalli.



Figure 5: DRIED FRUIT PULP

Preparation of Citrullus colocynthis fruit extract:

The collected fruits are cut and their pulp is separated which are then shade dried at room temperature (28^o-35^o C) for about 1 week and then coarsely grinded.

About 30gms of coarse powder of the fruit pulp was taken in a Soxhlet apparatus and was extracted using ethanol as a solvent. The temperature was maintained at around 80^o C for 24 hours. The extract

obtained was then concentrated by evaporating the ethanol. This gives the crude extract of *Citrullus colocynthis* fruit pulp.



Figure 6: SOXHLET EXTRACTION OF CITRULLUS COLOCYNTHIS FRUIT PULP

IV. PREFORMULATION STUDIES:

1. ANTIFUNGAL ACTIVITY OF CC EXTRACT:

The *Citrullus colocynthis* extract demonstrated marked antifungal activity in a concentration-dependent manner against *Candida albicans*. Maximum inhibition was recorded at 500 µg/ml, where *Candida albicans* showed inhibition zones comparable to those of the standard antifungal agent Amphotericin B.

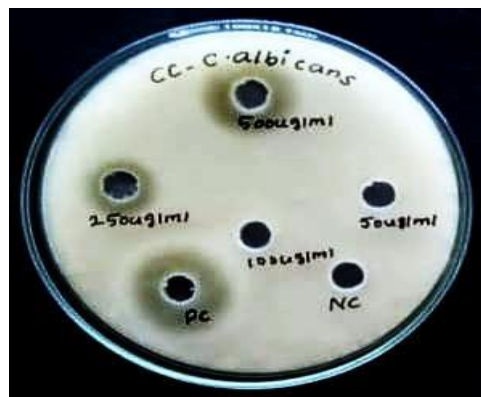


Figure 7: EFFECT OF CITRULLUS COLOCYNTHIS EXTRACT AGAINST CANDIDA ALBICANS.

NOTE: Since 500 µg/ml of the extract gave best antifungal activity it was decided to incorporate 1ml (~1mg) of crude extract was decided to be incorporated in the emulgel.

2. DRUG – EXCIPIENT COMPATIBILITY STUDY:^[15]

FTIR spectra of the *Citrullus colocynthis* extract was compared with spectra of prepared optimized and best emulgel formulation F1. The specimen was measured in an automatic recording IR spectrophotometer (Perkin Elmer – FTIR

spectrophotometer) mixed with Potassium bromide (KBr). A thin disc was prepared under anhydrous conditions from a powder containing about 1mg of material and 10- 100mg potassium bromide, using a mould and press. The range of measurement was from 4000 to 667 cm^{-1} (or 2.5 to 15 μm^{-1}) and the spectrum was recorded within three minutes.

V. FORMULATION OF CITRULLUS COLOCYNTHIS EMULGEL:

Table 1: DIFFERENT FORMULATIONS OF CITRULLUS COLOCYNTHIS EMULGEL

S.NO	INGERDIENTS	F1	F2	F3
1.	<i>Citrullus colocynthis</i> extract	1 ml	1 ml	1 ml
2.	Carbopol 934	0.5g	0.75g	1g
3..	Triethanolamine	Few drops	Few drops	Few drops
4.	Distilled water	q. s	q. s	q. s
5.	Tween 80	3 ml	3 ml	3 ml
6.	Liquid paraffin	15 ml	15 ml	15 ml
7.	Span 80	2 ml	2 ml	2 ml
8.	Methyl paraben	0.15 g	0.15 g	0.15 g
9.	Propylene glycol	2.5 ml	2.5 ml	2.5 ml
10.	Peppermint oil	1 ml	1 ml	1 ml

Formulation of Gel:

Three different formulations are prepared by varying only the concentration of the gelling agent which is Carbopol 934.



Figure 8:CARBOPOL 934 GEL

Accurately weighed quantities of Carbopol 934 namely 0.5g, 0.75g and 1g was taken and soaked in 50 ml of luke warm distilled water for 24 hours.

After 24 hrs the mixture was added with few drops of triethanolamine and mixed thoroughly using a glass rod or a magnetic stirrer at 100-150 rpm until a clear, transparent gel is formed with no lumps.

Formulation of Emulsion:

• Preparation of the oil phase:

The oil phase of the emulsion was prepared by dissolving 2 ml of Span 80, 0.15 g of methyl paraben and 2.5 ml of propylene glycol in 15 ml of liquid paraffin and heating upto 70⁰ C in a china dish.

• Preparation of the aqueous phase:

The aqueous phase of the emulsion was prepared by dissolving 3 ml of Tween 80 and 1 ml of *Citrullus colocynthis* extract in 30 ml of distilled water and heated in a separate china dish.

• Preparation of the emulsion:

The emulsion was prepared by slowly mixing the two phases (oil phase added to the aqueous phase) with constant stirring until stable emulsion is formed.



Figure 9: CITRULLUS COLOCYNTHIS EMULSION

Preparation of the emulgel:

The prepared gel and emulsion are blended and mixed together in 1:1 ratio using a magnetic stirrer at a speed of 200-250 rpm until a uniform, homogenous and stable emulgel is formed.



Figure 10: EMULGEL FORMULATIONS F1, F2 AND F3

VI. EVALUATION PARAMETERS:

The formulated emulgel was subjected to the following evaluation tests:

$$\text{Spreadability} = \frac{M}{T} \times L$$

1. **PHYSICAL EVALUATION:**^[16]

• **COLOUR:**

The colour of all the three formulations was checked against a white background.

• **ODOUR:**

The odour of the emulgel was checked by smelling aqueous solution of water.

• **GREASINESS:**

The greasiness was checked by applying the gel on the skin.

• **HOMOGENITY:**

Homogeneity of emulgel was checked by visual observation.

2. **pH TEST:**^[17]

pH of the prepared emulgel was measured using a digital pH meter. 1g of emulgel was dissolved in about 100 ml of distilled water and left for 2 hours. After certain time interval, the pH was tested by dipping the electrodes in the emulgel solution.

The measurement of pH was done for all the three formulations.

3. **DETERMINATION OF VISCOSITY:**^[18]

A Brookfield viscometer's spindle No. 7 was used to measure the viscosity of emulgel compositions. The required volumes of emulgel were added to a beaker, and the viscosity was measured at 100 rpm.

4. **SPREADABILITY:**^[19]

A unique arrangement was created in order to investigate the spreadability of formulations. The duration in seconds it takes for two slides to separate from material positioned between them when a specific load is applied is known as spreadability. The spreadability improves with a shorter time required to separate the two slides.

Two 7.5 x 2.5-centimeter glass slides were chosen. The formulation was put on top of a 500 mg slide whose spreadability needed to be ascertained. In order to position formulation between the two slides, this slide was positioned over the other slide. Weight (100 gm) was placed on the upper slide because the formulation between the two slides was continuously pushed to form a thin layer.

The lower slide was fixed on the cardboard support of the apparatus using a double side sticky tape and the upper slide was tied to a string. To this string load (20 gm) could be applied with the help of a simple pulley or tied to the other end.

The time it took for the upper slide to move 7.5 cm and separate from the lower slide was recorded under the weight-applied direction. The average of these results was computed for each formulation after the experiment was done three times.

Where,

M= Weight which is tied to the upper slide (20gm)

L= Length taken of glass slide (7.5cm)

T= Time taken (seconds).

5. **SWELLING INDEX:**^[19]

The method used to determine the swelling index of the prepared emulgel involves taking 1g of the gel on porous aluminum foil and placing it separately in a 50 ml beaker with 10 ml of 0.1 N NaOH. After

being reweighed, samples were withdrawn from beakers at various intervals and placed in a dry location for a while.

Swelling index is calculated as follows:

$$\text{Swelling index (SW\%)} = \frac{[W_t - W_0]}{[W_0]} \times$$

Where,

W_0 = Initial weight of emulgel at zero time

W_t = Weight of swollen emulgel after time t

(SW) % = Percent swelling Index.

6. UV- VISIBLE SPECTROPHOTOMETRIC ANALYSIS OF *CITRULLUS COLOCYNTHIS* EXTRACT:

UV-Visible spectrophotometric analysis of the crude extract was carried out to identify a suitable and reproducible wavelength for quantitative estimation. The extract was scanned over an appropriate wavelength range using phosphate buffer (pH-6.8) as blank, and a reproducible wavelength exhibiting maximum absorbance was selected for further analysis.

Based on the spectral characteristics and reproducibility of absorbance, wavelength was employed for the preparation of calibration curves and for subsequent in vitro release studies.

7. CALIBRATION CURVE OF *CITRULLUS COLOCYNTHIS* EXTRACT:

A stock solution of crude extract (10 $\mu\text{g/mL}$) was prepared by dissolving 0.5 mg of concentrated extract in 50 mL of phosphate buffer. From this stock solution, working standard solutions of 2, 4, 6, 8, 10 and 12 $\mu\text{g/mL}$ were prepared by suitable dilution with phosphate buffer. The absorbance of these solutions was measured at the selected wavelength using phosphate buffer as blank. A calibration curve was constructed by plotting absorbance versus concentration, and linearity was evaluated.

8. DRUG CONTENT DETERMINATION:^[17]

0.1g of emulgel was taken and was diluted with 10 mL phosphate buffer 6.8 and shaken to dissolve the drug in buffer. The solution was filtered through Whatman filter paper; 5 ml of the filtrate was taken out and diluted to 50 ml with buffer. The content of the drug was estimated spectrophotometrically and using slope and intercept using standard curve plotted at 290 nm.

9. IN-VITRO RELEASE STUDY:

Franz diffusion cell is used for the drug release study (A MODIFIED SETUP WAS USED).

A 250 ml beaker was used as a reservoir chamber and an open-end cylinder was used as a donor chamber. 500mg of emulgel was taken and applied on the film of cellophane evenly in an area fitting within the circumference of the cylinder end.

The cellophane film was clamped between the receptor and donor chamber of the diffusion cell with the help of a rubber band tied around the open-end tube.

Following this, the receptor chamber was filled with 50ml of freshly prepared phosphate buffer of pH 6.8 to solubilize the drug.

It was then stirred by a magnetic stirrer at 150-350 rpm and the temperature was maintained at 37 ± 0.2 . The samples were withdrawn at an appropriate time interval. They were then analyzed by using a UV visible spectrophotometer at a chosen wavelength having reproducible absorbance.^[20]

10. IN-VITRO ANTIFUNGAL ASSAY: PREPARATION OF CULTURE MEDIA:

The potato dextrose agar medium was prepared by dissolving 20 gm of potato infusion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 psi. pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

ASSAY PROCEDURE:

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr. culture of *Candida albicans* fungal strain and 500 $\mu\text{g/ml}$ concentration of samples F1, F2, F3 were added against *C. albicans*. The plates were then incubated at 28°C for 72 hours. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

The right amount of Potato dextrose agar was put into a 500 ml conical jar and then 250 ml of filtered distilled water was added. The Potato dextrose agar is fully broken down with heat. At 121°C and 15 psi, the autoclave was used for about 20 minutes. After that, it was left to cool to room temperature before the fungus *Candida albicans* was spread throughout the medium.

The medium was put into the correct Petri dish and it was then cooled until it hardened at room temperature. After that, a cork cutter with a 6 mm

diameter was used to measure out and pour the emulgel mixtures into the holes. After that, the Petri plates were put in incubators and kept at 28°C for 72 hours. After that, the zone of inhibition was measured to find out how big the inhibition zone was.

VII.RESULTS AND DISCUSSIONS:

1. PREFORMULATION STUDIES: DRUG – EXCIPIENT COMPATIBILITY STUDY:

FTIR analysis of the *Citrullus colocynthis* extract and its F1 emulgel formulation exhibited distinct

absorption peaks for **hydroxyl (O–H), carbonyl (C=O), and aromatic C=C groups**, suggesting the presence of phenolic, ketone, and aromatic compounds.

This confirms that the bioactive phytoconstituents were successfully retained in both the extract and the emulgel. FTIR spectroscopy revealed key functional groups—including hydroxyl, carbonyl, and aromatic C=C—indicative of phenolic, ketonic, and aromatic bioactive molecules, and these groups were retained in the optimized F1 emulgel, confirming chemical stability and efficient incorporation of the extract into the topical formulation.

FTIR Analysis of *Citrullus colocynthis* Extract:

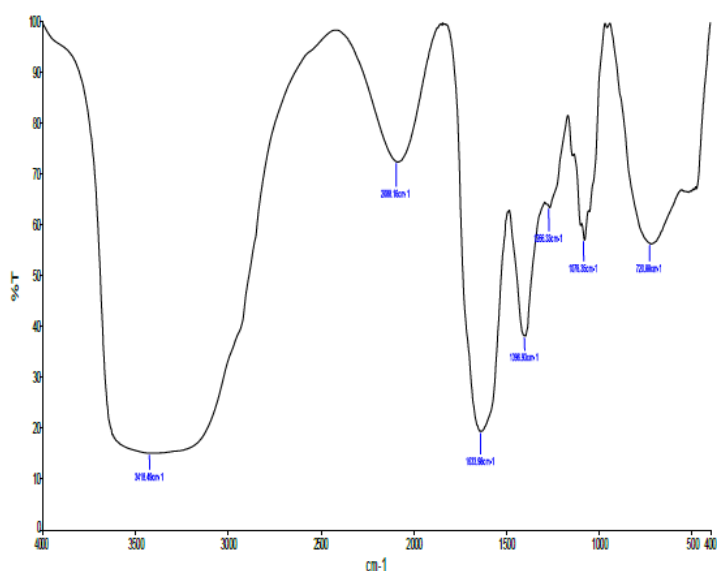


Figure 11: FTIR SPECTRUM OF *CITRULLUS COLOCYNTHIS* EXTRACT

FTIR Analysis of F1:

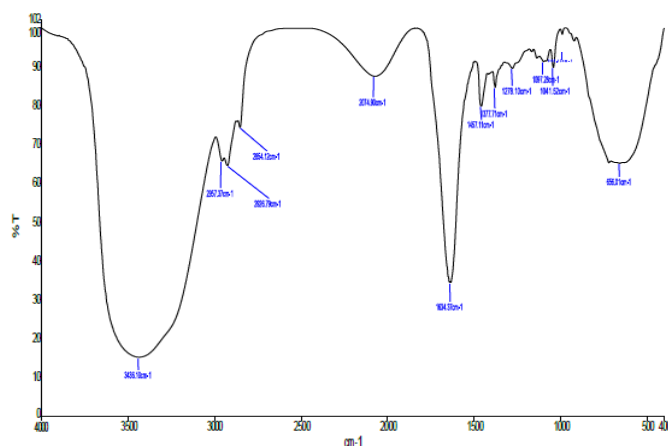


Figure 12: FTIR SPECTRUM OF F1

2. EVALUATION TESTS:

Three different formulations of *Citrullus colocynthis* emulgel was formulated with varying concentrations of the gelling agent Carbopol 934. The formulated emulgels were evaluated for various parameters using the procedures described in previous chapter. The results of the evaluation tests are discussed as follows:

1. PHYSICAL EVALUATION:

Table 2: PHYSICAL EVALUATION

FORMULATION	F1	F2	F3
COLOUR	Off-white/cream colour	Off-white/cream colour	Off-white/cream colour
ODOUR	Pleasant	Pleasant	Pleasant
GREASINESS	Non-oily	Non-oily	Non-oily
HOMOGENITY	Excellent	Good	Excellent
PHASE SEPERATION	None	None	None

2. pH TEST:

It was found that the pH of all formulations was in the range of pH 5.5 to 6 which indicates skin compatibility i.e., *Citrullus colocynthis* extracts emulgels can be applied to the skin without any discomfort or irritation. The results are presented in Table 3.

Table 3: CHARACTERISATION OF *CITRULLUS COLOCYNTHIS* EMULGEL FORMULATIONS FOR pH, VISCOSITY, SPREADABILITY

FORMULATION	pH	VISCOSITY	SPREADABILITY * (g.cm/sec)
F1	5.52	1264 cps	39.30 ± 7.10
F2	5.82	1337 cps	29.89 ± 5.63
F3	5.85	1395 cps	22.78 ± 4.32

*Values are expressed as mean ± SD

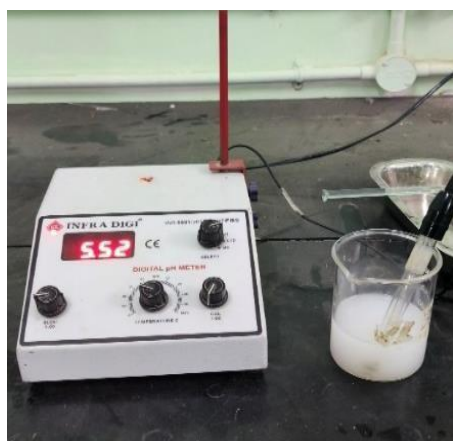


Figure 13: pH TEST

3. VISCOSITY/RHEOLOGICAL STUDY:

All the formulations exhibited viscosity values within an acceptable range for topical emulgel application. An **increase in viscosity was observed from F1 to F3, which may be attributed to the increasing concentration of Carbopol 934 gelling agent in the formulations.** Higher polymer concentration leads to enhanced intermolecular interactions and formation of a stronger gel network, resulting in increased resistance to flow. The results are presented in Table 3.

4. SPREADABILITY:

The spreadability of formulated emulgels was found to decrease with an increase in Carbopol 934 concentration. Formulation F1 containing 0.5g of Carbopol 934 showed the highest spreadability, whereas F3 having the highest Carbopol concentration of 1g showed lowest spreadability. The results indicate that Carbopol 934 concentration plays a significant role in controlling the rheological behavior and spreadability of emulgels.



Figure 14: SPREADABILITY TEST

5. SWELLING INDEX:

The swelling index was calculated based on the percentage increase in weight after immersion in the swelling medium upto 4 hrs. The results are presented in Table 3.



Figure 15: DETERMINATION OF SWELLING INDEX

The swelling index of all emulgel formulations increased progressively with time. Among the formulations, F3 exhibited the highest swelling index throughout the study period, reaching a maximum value of 198% at 4 hours. Formulation F1 showed moderate swelling (148%), while F2 demonstrated comparatively lower swelling (125%) at the end of 4 hours. (Table 4)

Table 4: % SWELLING INDEX OF THREE FORMULATIONS

TIME (in minutes)	SW% (F1)	SW% (F2)	SW% (F3)
0	0	0	0
30	7	23	26
60	37	46	67
120	56	82	109
180	92	114	144
240	148	125	198

Higher swelling facilitates better gel expansion, which is advantageous for topical drug delivery systems as it may promote drug diffusion.

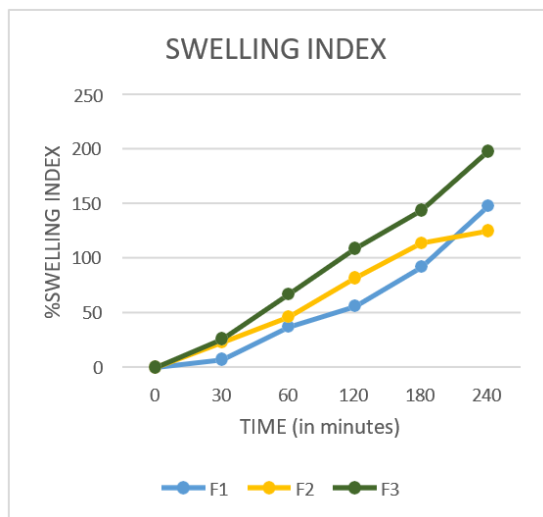


Figure 16: COMPARATIVE % SWELLING INDEX OF THREE FORMULATIONS

6. UV- VISIBLE SPECTROPHOTOMETRIC ANALYSIS OF CITRULLUS COLOCYNTHIS EXTRACT:

UV-Visible spectrophotometric analysis of the crude extract was carried out to identify a suitable wavelength for quantitative estimation.

Among the observed absorption regions, a reproducible and stable absorbance was noted around **290 nm**.

The selection of 290 nm was further supported by the fact that several flavonoids, phenolic and aromatic phytoconstituents are known to absorb in this region. Hence, 290 nm was considered an appropriate analytical wavelength for quantitative estimation and was subsequently employed for calibration curve development and in vitro release studies.

7. CALIBRATION CURVE OF CITRULLUS COLOCYNTHIS EXTRACT:

The calibration curve of the crude extract constructed at 290 nm showed a linear increase in absorbance with increasing concentration, indicating compliance with Beer-Lambert's law.

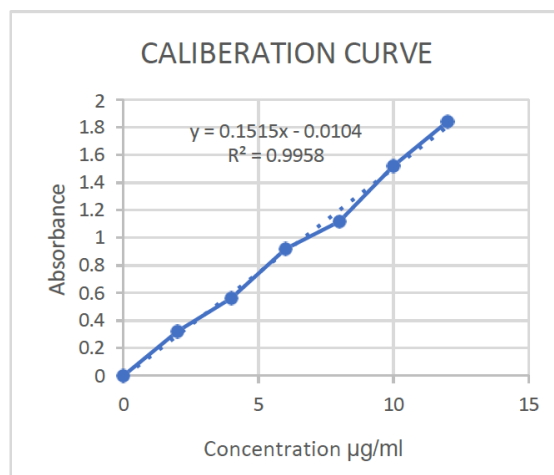


Figure 17: CALIBRATION CURVE OF CITRULLUS COLOCYNTHIS EXTRACT

Linear regression analysis demonstrated good linearity, confirming the suitability of the selected wavelength for quantitative estimation.



Figure 18: WORKING STANDARD SOLUTIONS FOR CALIBRATION CURVE

The established calibration curve was successfully applied for extract content determination and in vitro release studies, indicating that the developed UV spectrophotometric method is simple and reproducible.

8. DRUG CONTENT DETERMINATION:

Drug content analysis was carried out using UV-Visible spectrophotometry at 290 nm employing crude extract as the reference standard. The total extract content of emulgel formulations ranged from 0.742 to 1.237 mg/g, with F1 showing the highest extract content.

Table 5: DRUG (TOTAL EXTRACT) CONTENT:

FORMULATION	ABSORBANCE (290 nm)	TOTAL EXTRACT CONTENT (mg/g)
F1	0.177	1.237
F2	0.153	1.078
F3	0.102	0.742

The observed variation in extract content among formulations may be attributed to differences in polymer concentration affecting viscosity and extract distribution within the gel matrix.

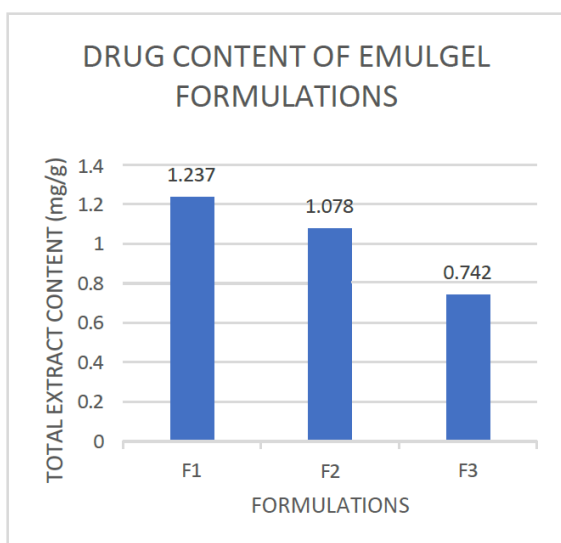


Figure 19: COMPARATIVE DRUG CONTENT OF THREE FORMULATIONS

9. IN-VITRO RELEASE STUDY:

The in-vitro release study showed a **controlled and sustained drug release** from all emulgel formulations over a period of 6 hours. The cumulative percentage drug release was calculated at predetermined time intervals and is depicted in FIG. Formulation F1 exhibited the highest cumulative drug release (58.80%), followed by F2 (40.42%) and F3 (34.15%).

The order of drug release was: **F1 > F2 > F3**.



FIGURE 20: MODIFIED FRANZ DIFFUSION APPARATUS



FIGURE 21: SAMPLES TAKEN FROM RESERVOIR CHAMBER

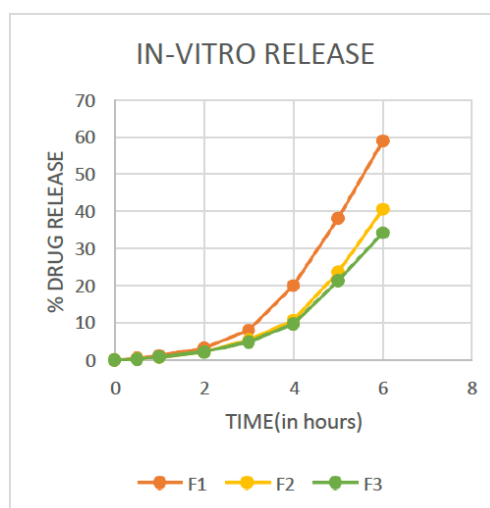
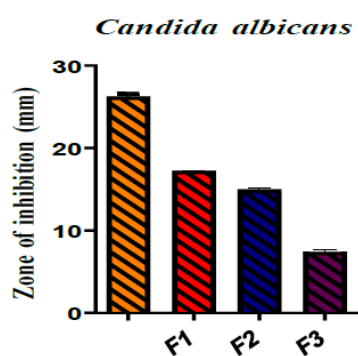
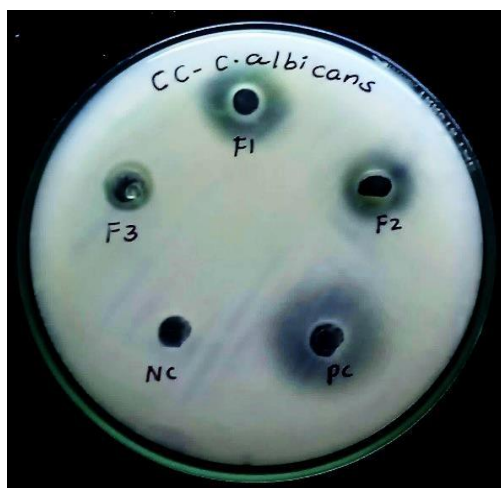


FIGURE 22: COMPARATIVE DRUG RELEASE OF THREE FORMULATIONS

9. IN-VITRO ANTIFUNGAL ASSAY:

The *Citrullus colocynthis* emulgel formulations (F1, F2, and F3) demonstrated antifungal activity against *Candida albicans*, with **F1 showing the greatest inhibition zone of**

17.25 ± 0.07 mm. In contrast, F3 exhibited the lowest activity, while the standard drug Amphotericin B produced the highest inhibition of **26.25 ± 0.35 mm.**



CC - F1, F2 and F3

Figure 203: COMPARATIVE ZONES OF INHIBITION OF F1, F2 AND F3

Table 6: ANTIFUNGAL ACTIVITY OF F1, F2 AND F3 AGAINST *CANDIDA ALBICANS*

S.NO	Name of the test organism	Zone of inhibition (mm) SD ±			
		F1	F2	F3	PC
1.	<i>Candida albicans</i>	17.25 ± 0.07	15 ± 0.14	7.45 ± 0.21	26.25 ± 0.35

SD-Standard Deviation, *Significance - p<0.05

VIII. CONCLUSION:

The present work successfully formulated and evaluated a topical antifungal emulgel containing *Citrullus colocynthis* fruit extract, establishing it as a promising herbal delivery system for superficial fungal infections.

Overall, F1 can be considered the optimized formulation, offering a suitable balance of spreadability, release profile and antifungal activity, and the study supports further in-vivo, stability and comparative studies to develop *Citrullus colocynthis* emulgel as a safe, patient-acceptable herbal antifungal product.

Future studies may focus on isolation, purification, and characterization of the specific antifungal bioactive compound(s) present in *Citrullus colocynthis*, which would allow more targeted and standardized therapy. Advanced formulation strategies such as nano-emulgels, vesicular carriers, or penetration-enhanced systems may be explored to further improve skin permeation and antifungal efficacy.

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