Original Research Article

Formulation and Evaluation of Calendula and Lavender Oil Based Topical Antifungal Gel

ABSTRACT

Aim: *Calendula officinalis* and *Lavendula angustifolia* are traditional medicinal plants that have antifungal activity. A combination of these two plants has not been known for its activity against fungus. The present study deals with the formulation and evaluation of herbal topical Antifungal gel containing essential oils of both plants.

Study design: Proto type research design

Place and Duration of Study: Smt. R. D. Gardi Pharmacy College, Nyara- Rajkot. January 2024 to April 2024.

Methodology: As 90% of skin infections are caused by *Candida albicans*, the antifungal activity of topical gel against this species was assessed. In this investigation, we created an antifungal herbal gel by combining carbopol 940 and other excipients with the essential oils of *Calendula officinalis* and *Lavendula angustifolia* in varying concentrations. For compatibility study, FTIR study would be done. Evaluation of Antifungal gel performed by using different tests likes, pH, Stability, Extrudability, Spreadability, Viscosity, Antifungal activity against *Candida albicance*, and *in-vitro* drug diffusion study.

Results: As a result, the formulation's physiochemical characteristics, *in vitro* antifungal efficacy, and stability analysis (stable even after 30 days) were assessed. Every herbal gel formulation with a pH of 5–7 had favorable outcomes for physiochemical measures. Out of all the formulations, batch no 4 exhibited superior release characteristics (98 %) and zone of inhibition in comparison to the other formulated batches. The drug release profile of batch 4 showed great results.

Conclusion: This is the first study on the scientific evaluation of *Calendula officinalis* and *Lavendula angustifolia* essential oil as a gel for antifungal activity. Thus this study reveals both good antifungals and *in-vitro* drug release; their essential oil may be formulated as Topical Gel Antifungal with satisfactory physicochemical parameters.

Keywords: Calendula officinalis, Lavendula angustifolia, Candida albicans, Essential Oil, Topical Antifungal Gel.

1. INTRODUCTION OF ANTIFUNGAL TOPICAL GEL

A gel is a semi-solid, three-dimensional matrix created by the permeability of a solvent into a network of intertwined polymer chains or by an interspersed system of colloidal particles. A gelator, also known as a gelling agent, is added to the solvent and active component mixture to create pharmaceutical gels. Small compounds with low molecular weight or polymers can be utilized as gelators in gel formulation. As a dispersion media, the solvent might be an aqueous, organic, inorganic, or a combination of solvents. Topical gels are applied to the skin to act as a contact or transport medium for active medications. The gel's three-dimensional mesh entangles the active drug molecules. Gels differ from conventional

dosage forms in a few key ways, including stiffness, rheology, swelling, aging, and syneresis. Gels can be categorized based on a range of factors, including their physical makeup, kind of colloidal phase, and type of solvent utilized. Due to its advantages over creams and ointments, topical gels are a topical medication delivery dosage form that is frequently employed in cosmetics and treatments for skin ailments. They are created by combining a gelator, solvent, active medication, and other excipients. [1-21]

1.1 Introduction of calendula essential oil

Figure 1: *Calendula officinalis*

Calendula officinalis is a flowering plant in the daisy family asteraceae. Its florets are edible. They are often used to add color to salads or added to dishes as a garnish instead of saffron. The essential oil of calendula is high in triterpenoid content which heals dry skin, eczema, and hemorrhoids. *Calendula officinalis* is widely cultivated and can be grown easily in sunny locations in most kinds of soils. [22-26]

1.1.1 Phytoconstituents

The plant has flavonoids, triol triterpenes, xanthophylls, esquiterpene glycosides, saponins, and volatiles, terpenoids, flavonoids, triterpeneol esters, steroids, phenolic compounds, carotenes, triterpenoids, essential oils, quinones, fatty acids, minerals, saponins, carbohydrates, sterols, and tocopherols, among other classes of chemical compounds. Calendula officinalis flowers are orange-colored because it is rich in carotenoids. The oil is rich in volatile oils, sterols, beta carotene, rutin, lupeol, narcissin, and calendulin. Calendula officinalis contains flavonoids, including quercetin, which has strong wound-healing properties. [27, 28]

1.1.2 Medicinal benefits of calendula oil

Calendula oil works wonders as a moisturizer for all skin types. The oil relieves the damaged region and does wonders for dry or split skin. Calendula essential oil helps to cure hemorrhoids, eczema, and dry skin. It cures mouth infections and lowers body temperature. One can consume calendula oil internally. On the outside, the oil is utilized in soaps, lip balms, lotions, and creams. According to studies, the oil known as "the mother of the skin" is highly regarded by homeopathic herbalists. It exhibits pharmacological activity like anti-inflammatory, antioxidant activity, cytotoxic and anti-tumor activity, wound-healing activity, antimicrobial activity, antibacterial activity, and antifungal activity. [28-44]

1.1.3 Pharmacological activity

Calendula oil shows pharmacological activities like antiviral, antifungal, antioxidant, antiseptic, antitumor, anti-inflammatory, antioxidant, hepatoprotective, and antidiabetic activity. [30-44]

1.2 Introduction of Lavender essential oil

Figure 2: *Lavendula angustifolia*

The plant's scent is produced by shimmering oil glands embedded in the microscopic plant hairs, or trichomes, that cover the stems, leaves, and flowers of the plant. Taxonomic classification of *lavendula angustifolia* is: scientific name: lavandula, family: lamiaceae, subfamily: nepetoideae, kingdom: plantae, order: lamiales, tribe: ocimeae.

1.2.1 Medicinal benefits of lavender oil

Lavender may have antifungal effects, as shown by numerous research. Studies suggest that the essential oil of lavender may help inhibit the growth of certain fungi, such as candida albicans. The oil can also be used to treat athlete's foot and ringworm. The pharmacological actions of lavender angustifolia oil are well-known, and they include sedative, antidepressant, healing, antiseptic, antifungal, relaxing, and antiemetic qualities. [45-47]

1.2.2 Phytoconstituents

The main component of lavender essential oils is monoterpenes.the most common monoterpenes are linalool and linalyl acetate. Volatile oils like linalool, limonene, perillyl alcohol, linalyl acetate, cis-smine, terpene, coumarin, tannin, caffeic acid, and camphor. The main constituents that give antifungal activities are linalyl acetate and linalool. [48-61]

1.2.3 Pharmacological activity

The plant shows pharmacological activities like antimicrobial, anti-inflammatory, antinociceptive properties, anxiolytic, antioxidant, and antifungal activity [62-64]

2. MATERIAL AND METHODS

2.1 API and Excipients

API (Active pharmaceutical ingredients): Calandula oil and Lavandula oil.
Excipients: Carbopol 940, Glycerine, Propylene glycol, DM DM Hydantoin, PEG-40 Hydrogenated Castor oil, Diethylene glycol monoethyl ether, Vitamin E, Triethanolamine [65-74]

2.2 Collection of Essential Oil

2.2.1 Collection of Calendula Oil:

Veda oil Calendula Essential Oil, Marigold Essential Oil | ACTIZEET, Calendula Oil Pure & Natural Carrier Oil, Deve Herbs Pure Calendula Oil, Ks Essentials *Calendula Officinalis* From above mentioned different branded calendula oil products we have selected the Ks

Essentials *Calendula Officinalis* as it is good quality and low-cost product compared to other products. Ks Essentials 100% Pure Calendula Essential Oil Pure Natural & Undiluted for Skin Care & Hair. [72]

Table 1: Certificate of Analysis of Calendula Essential Oil

Certificate of Analysis		
Product: Calendula Oil		
Shelf Life: 2 Years		
Test	**Standards**	**Results**
Botanical Name	*Calendula Officinalis*	Complies
Appearance & Color	Clear yellow to orange liquid	Complies
Odor	Mild characteristic odor	Complies
Refractive index at 20°C	1.450 - 1.510	1.467
Acid Value	Max 4.0	1.04
Specific gravity at 25°C	0.905 - 0.923	0.920
Iodine Value	112 – 145	123.20
Peroxide Value	Max 20.0	3.36
Saponification Value	190-200	197
Unsaponifiable Matter	Max 1.50%	1.0%
Water	Max 0.1%	Complies
Solubility	Very slightly soluble in Alcohol and completely miscible With light petroleum	
Storage	In a well-fitted container, in a cool and dark place.	

2.2.2 Collection of Lavender Essential oil

Vagad's khadi Herbal Lavender Essential Oil is used for this preparation.

2.2.3 Collection of Excipients

Carbopol 940, glycerine, propylene glycol and triethanolamine were collected at Smt. R. D. Gardi Pharmacy College, Nyara-Rajkot. PEG-40 Hydrogenated Castor Oil and DM DM Hydantoin were bought online from purenso select official website. Diethylene Glycol, Monoethyl Ether were bought from CHEMDYES CORPORATION "Rasayan Ghar" Kotharia Naka Chowk, Near Soni Bazaar RAJKOT - 360001 (Gujarat).

3. FORMULATION AND EVALUATION OF ANTIFUNGAL GEL [73]

3.1 Gel Formulation Ingredients

Formulation of topical gels is determined by important factors such as appearance, odor, Spreadability, Extrudability, viscosity, pH, texture, microbial contamination potential and bioavailability. The components of the vehicle should serve to make the skin surface more penetrable to the drug. Characteristics of the gel such as consistency and viscosity are affected by formulation design. Consistency and viscosity affect the adhesion and retention property of the gel, and are important in ensuring the gel is retained at the site of application and effective delivery of the drug. The ingredients in topical gel formulation can be broadly categorized into four types: gelator, solvent, drug, and excipients.

3.1.1 Gelator

Gelators serve as stabilizers and thickeners, thickening the gel solution while simultaneously

maintaining the gel's flexible nature. When dispersed through the solvent as a colloid, gelators offer a stable internal structure to the gel. Gelators are usually chosen based on their affinity for the solvent and the purpose of the gel. The nature of the gelators used determines the rigidity of the gel. Natural gelators include aloe vera gel, tragacanth, gelatin, collagen, and guar gum; semi-synthetic gelators include methylcellulose and other cellulose derivatives; while synthetic gelators include carbomers, polyvinyl alcohol, polyethylene, and its copolymers.

3.1.2 Solvent

Solvents are usually chosen based on the applications of the gel. They can be hydrophilic, lipophilic, or organic. Individual solvents can be used alone or as a mixture.[5] Some examples of solvents include purified water, glycerin, glycols, alcohols, sucrose, toluene, and mineral oils.

3.1.3 Drug

Topical delivery is often used for drugs that are easily degraded in the GI tract, or are highly susceptible to hepatic first pass effect. Even if the drug has to be administered for long periods or can induce adverse drug reactions in parts of the body other than the target location, it can still be formulated as a topical gel.

3.1.4 Excipients

Excipients are materials inert to the drug, which are added into dosage forms to improve the overall quality of the dosage form. Some examples include antioxidants, sweetening agents, stabilizers, dispersing agents, penetration enhancers, buffers and preservatives. Penetration enhancers are excipients that can increase skin permeability. Many classes of excipients can be used as penetration enhancers, such as glycerin, sulfoxides and related analogues, pyrrolidines, fatty acid and ethanol, surfactants etc. Buffers can be added to control the pH of aqueous or hydroalcoholic based gels. Preservatives are important for their antimicrobial action, and are especially important in formulation of hydrogels. Antioxidants are used to prevent gel ingredients from being oxidised. When choosing the antioxidant to be used, it is important to consider the nature of the solvent. Since the solvent of most gels are aqueous in nature, water-soluble antioxidants are more commonly used. Sweetening agents are only used in gels that are designed to be used in the oral cavity such as dental gels.

3.2 Gel Preparation Methods

The process of gel formation involves finding a balance between the concentrations of the gelator and the solvent. When adding a gelator to the solvent, the mixture remains in liquid state. As the concentration of the gelator increases to a certain critical concentration, gelation occurs through swelling to form the semi-solid gel. Further increasing the concentration of the gelator beyond the gelling point will increase gel viscosity.

The exact gelling point varies depending on the properties of the gelator and the solvent, such as structure uniformity, molecular weight of the polymer, and flexibility of the polymer chain. Generally, gels are prepared by first dissolving the soluble excipients in the solvent. The gel is allowed to settle for one to two days before the final consistency of the gel can be reached. The exact method of preparing gels depends on the properties of the formulation ingredients.

3.3 Advantages of Topical gels

The texture of topical gels is less greasy as it contains a higher proportion of water compared with cream and ointment. These gels have an excellent spreading property and cooling effect due to solvent evaporation and also has a higher retention time on the skin.

Topical gels are more stable than creams and ointments and can adhere well to the site of application. They form an occlusive layer on the application site that can act as a form of

protection. They can be washed off easily and are nontoxic due to their unique composition and structure. They have minimal side effects due to their localized effect. Topical gels are convenient and easy to apply. The topical mode of action of topical gels is also non-invasive. These favorable factors of topical gels improve patient compliance and tolerability. The formulation and manufacturing processes of topical gels are relatively simpler and more cost effective than other semisolid dosage forms. The release profile of the gel can be modified by altering the properties of the gelator, allowing for continuous drug delivery. Topical gels are also eco-friendly, biocompatible, and biodegradable.
The topical dosage form allows stable and continuous drug delivery to the site of application while having a faster drug release than ointments and creams. All these can increase the drug's bioavailability in the body. Better application properties and stability in comparison to cream and ointments.

3.4 Method for Formulation of Gel [73]

3.4.1 Dispersion Mehod

Gelling agent was dispersed in water with stirring at 1200 rpm for 30 min. Drug was dissolved in non-aqueous solvent with preservative, this solution was added in above gel with continuous stirring.

Phase A

Take water in a beaker, add Carbopol 940 & dissolve completely. Add glycerine on continue stirring then add PG. Mix well. Add DM DM Hydantoin on continue stirring.

Phase B

In another beaker, weigh PEG40 hydrogenated castor oil & Add Diethylene glycol monoethyl ether. Mix well until it becomes transparent. Add lavender oil, calendula oil & vitamin E one by one. Mix well until clear. Add phase B to phase A. Mix well. Check pH & adjust with Triethanolamine. The transparent gel is formed.

3.5 Evaluation of Antifungal Gel [74-78]

3.5.1 FTIR (Fourier Transform Infrared Spectroscopy)

Interactions between an API and its excipients impact the API's stability, chemical structure, and bioavailability. As FTIR spectroscopy detects vibrational shifts that may indicate possible intermolecular interactions between dosage components, it is helpful not only for studying the behavior of solid-state APIs and their excipients but also as a compatibility screening tool.

3.5.2 FTIR Analysis

FTIR study was performed and FTIR spectra were recorded on FTIR spectrophotometer with IRSpirit_SHIMADZU-IR-Instrument1 at Public Testing Laboratory, GTU, Gandhinagar, Gujarat, India and Instrumental Facility Section Department of Pharmaceutical Sciences Saurashtra University Rajkot-India by MS method. FTIR spectral data were recorded and functional group stretching and bending wavelength were matched with literature published data.

3.5.3 Physical appearance

The physical attributes (color, look, and feel), organoleptic parameters (phase separation, and liquefaction), pH, viscosity, Spreadability, and oil content were also observed at various intervals for 30 days.

Appearance - Colour is important for patient compliance. The prepared gels were inspected visually for clarity, colour, and presence of any particles.

Homogeneity - All developed gels were tested for homogeneity by visual inspection after the gels had been set in the container. They were tested for their appearance and the presence of any aggregates.

pH

pH 1.0 g gel was accurately weighed and dispersed in 100 ml purified water. The pH of the dispersion was measured using a digital pH meter, which was calibrated before use with a standard buffer solution at 4.0, 7.0, and 9.0. The measurements of pH were done in triplicate and average values were calculated.

Viscosity

Brookfield DV-III ULTRA PROGRAMMABLE RHEOLOGICAL RHEOMETER and DV-II+ Pro viscometer and was used for the determination of viscosity. Gel samples were placed at room temperature for 30 min. Then, they were poured into an apparatus container. Number 64 spindle was attached then viscosity was determined at 25°C and 100–250 rpm. The results were reported on average after triplicate experiments.

Spreadability

One of the criteria for a topical formulation to meet the ideal qualities is that it should possess good Spreadability. It is the term expressed to denote the extent of the area to which formulation readily spreads on application to the skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. To determine the Spreadability of formulation, 0.5 g of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted.

Extrudability

To determine Extrudability a closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, the formulation extruded until the pressure dissipated. The weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 Seconds was determined. The average extrusion pressure in g was reported.

Stability

The stability study was assessed by storing the formulation at different storage conditions and at room temperature (25-28°C).

3.5.4 Antifungal Activity

For a variety of causes, the quantity of antifungal susceptibility testing (AFST) carried out has increased recently. In recent decades, there has been a rise in the number of patients with risk factors for invasive fungal infection (IFI), such as deep immunosuppression, prolonged use of broad-spectrum antibiotics, and implanted medical devices.

This has also led to an increase in the incidence of IFIs. Clinical microbiology labs frequently use AFST as a technique to help choose the best antifungal medication. By figuring out the medication concentration needed to inhibit an organism to a certain degree—referred to as the minimum inhibitory concentration, by definition, gives an *in vitro* assessment of susceptibility and resistance. Testing for antifungal susceptibility may be useful in ways other than choosing an antifungal medication for a certain patient.

Procedure: The antifungal activity of prepared formulation was tested at Neugen Unipath-Health Checkup / Pathology lab/ Diagnostics center in Rajkot against *candida albicans* by agar diffusion method.

3.5.5 *In vitro* Drug Diffusion Study

The Franz diffusion cell was mainly used to assess the stability and permeability of formulations, including transdermal and topical. The in-vitro and ex-vivo drug release from topical treatments such as creams, ointments, liposome formulations, and gels can be accurately measured using the simple diffusion cell assay. It provided very important viewpoints on the interactions between the skin, medication, and formulation.

Furthermore, it is utilized for quality control and toxicity testing.

Procedure: *In vitro* drug release was determined using a Franz diffusion cell and synthetic membrane. 1 g of the test sample was dispersed uniformly on the membrane surface; finally, it was fixed on the cell. cell receiver phase contained phosphate buffer, pH 6.8. The

temperature of 37°C was controlled by a pumped water bath circulating between 2 shells encompassing the chamber.
Franz diffusion cell was placed at receiver phase space by a magnetic stirrer to obtain sink conditions. This set was also put on a magnetic mixer then the cell mouth was covered by parafilm to avoid evaporation from the donor phase. A volume of 1 ml samples was taken at specified time intervals. After each sampling, the aliquots were replaced by fresh phosphate buffer, pH 6.8 subsequently to gain the same volume of receiver phase during the experiment. The test was repeated three times for each sample, and the absorbance was measured in a UV Spectrophotometer.

4. RESULTS AND DISCUSSION

4.1 FTIR Study

Based on FTIR spectral data of essential oils as well as excipients, it is confirmed that the characteristic peaks of essential oils are preserved in the gel formulations, showing the absence of any type of interaction among formulation constituents.

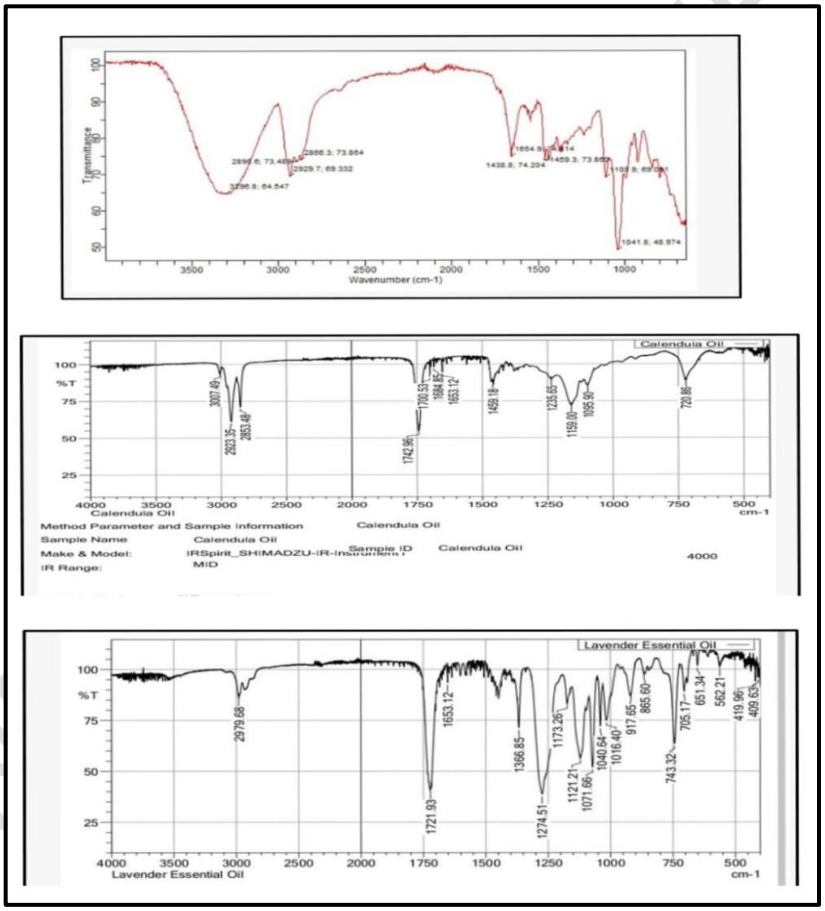

Figure 3: FTIR analysis

4.2 Formulations of Different Batches

Table 2: Formulas of Different Batches of Antifungal Gel

Ingredient	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Carbopol 940	0.5 %	0.6%	1%	1%	1.5%	1.5%	2%
Glycerin	3%	1.6 %	2%	2%	2%	3%	3%
Propylene glycol	5%	1.6%	3%	5%	4%	5%	3%
DM DM Hydantoin	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
PEG40 hydrogenated castor oil	4%	5%	3%	5%	5%	5%	4%
Diethylene glycol monoethyl ether	4%	5%	2%	4%	4%	5%	4%
Calendula essential oil	0.5%	1%	1%	1%	1%	1%	1%
Lavender essential oil	0.5%	0.5%	1%	1%	1%	1%	1%
Vitamin E	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s

By using all the above Formulas Antifungal Gel was prepared in different batches shown in Figure 4.

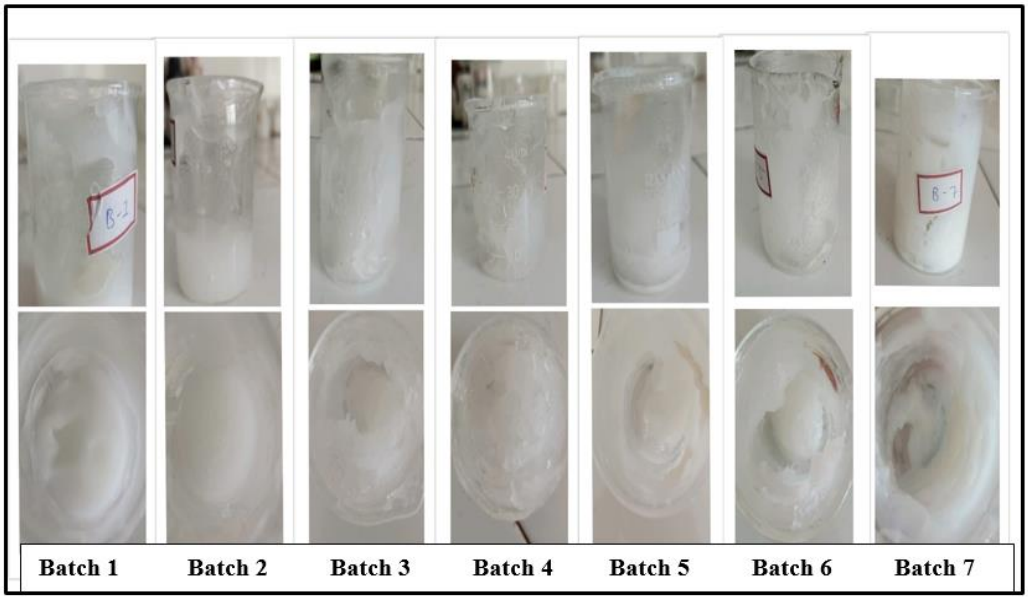

Figure 4: different batches of formulations

4.3 pH and Viscosity of All Formulations

Batch no.	pH	Viscosity (cP)
1	5.5	2983
2	5.7	1082

Table 3: pH & Viscosity of all formulations

3	6.8	1297
4	6.2	1987
5	5.9	2787
6	6	1095
7	6.9	1276

The pH of all formulations was found to be in the range of 5-7 (Table 3), which was near to skin pH. Batch no. 1,2,5 and 6 showed a pH near to range of skin pH 5.

4.4 Spreadability of All Formulations

Batch 1, 2, and 4 showed better Spreadability than other batches.

4.5 Extrudability of All Formulations

Extrudability study shows batches 1,2 and 4 have good Extrudability.

4.6 Stability of All Formulations

Stability study was done at 0, 15^{th,} and 30th days by examining all formulations of any visible and above-performed evaluation parameters regarding changes. All the formulations were found stable as none of them showed any changes in parameters. Batch no. 4 was found to be more stable than other formulations.

4.7 Antifungal Activity Against *Candida Albicans*

Antifungal activity against *candida albicans* was performed by using the agar diffusion method of batches 1,2 and 4. Batch 2 showed slight activity at different concentrations as shown in the figures. Batch 4 was found to be a better antifungal topical gel as results shown in the figures.

Figure 5: Antifungal activity of Batch 4

Table 4 Zone of inhibition in *Candida albicance* species

Batch no	Concentration (µl)	Zone of inhibition
1	100	0
2	200-400	2.5 mm
4	100-200	5 mm

4.8 *In vitro* Drug Diffusion Study

Table 5: Drug Release of Batch 4

Time (hours)	Drug Release %
0	0
1	30
2	45
3	57
4	78
5	89
6	98

Drug release of batch 4 formulation was carried out by using a Franz cell diffusion study for 6 hours and results were determined by using a UV spectrophotometer at 413 nm wavelength.

5. CONCLUSION

The risk of fungal infections to human health is still there and getting worse. This innovative herbal topical antifungal gel, which contains essential oils of *calendula officinalis* and *lavender angustifolia*, is biocompatible and provides an alternative form of treatment for fungal infections. The gel is intended to reduce the adverse effects of standard antifungal drugs that are commercially accessible, including drug resistance and toxicity. To evaluate the patients for improvements in clinical signs and symptoms, more clinical research must be done. In summary, all evaluation parameters showed positive results for the topical antifungal gel based on calendula and lavender oil, which was prepared with carbopol 940 and other excipients. This suggests that the gel is efficient against pathogens. Out of all the formulations, batch number four demonstrated excellent results and was subsequently selected for a study on drug release, revealing drug release within six hours. To improve patient consistency, topical medications will be utilized more often in the future.

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