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FORMULATION AND EVAULATION OF ANTIMICROBIAL EMULGEL BY USING FLEX SEED OIL AND THYMUS SEED OIL

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ABSTRACT

Introduction: Antimicrobial resistance has posed a significant challenge to healthcare systems worldwide, necessitating the exploration of novel antimicrobial agents and delivery systems. Among these, emulgels have garnered attention as versatile platforms for drug delivery due to their ability to combine the benefits of both emulsions and gels. Emulgels offer advantages such as enhanced stability, prolonged release of active ingredients, and improved stability.

Aim: This study aimed to formulate and evaluate an antimicrobial emulgel incorporating flaxseed oil and thyme seed oil. The emulgel was prepared using a standard emulsification process, followed by the incorporation of a suitable gelling agent to achieve the desired consistency.

Methods: The antimicrobial activity of the emulgel was assessed using agar well diffusion method against common pathogenic bacteria such as Staphylococcus aureus or Pseudomonas aeruginosa and Escherichia coli. The physical properties of the emulgel,including pH**,**viscosityand Spreadability,extrudability,were evaluated to ensure its suitability for topical application. Results demonstrated that the emulgel exhibited significant antimicrobial activity, with zones of inhibition comparable to standard antibiotics.

Results: The formulated emulgel showed optimal pH, (**6.8 ± 0.081 to 7 ± 0.047),** acceptable viscosity, (**14200 ± 100 to15100 ± 360.55),** and good Spreadability (**5.30 ± 0.016**, **to 5.60 ± 0.016**) extrudability to**13 ± 0.816 to14 ± 0.471**)antimicrobial activity (**13mm to22.35mm)** indicating its potential for effective skin application. **Conclusion:** The emulgel containing flaxseed oil and thyme seed oil possesses promising antimicrobial properties and favourable physical characteristics, making it a potential candidate for topical antimicrobial therapy. Further studies are warranted to explore its stability and clinical efficacproved patient compliance.

Keywords: *Flaxseed oil, Thyme seed oil, Antimicrobial emulgel, Staphylococcus aureus and Escherichia coli*

INTRODUCTION:

In recent years, the emergence of antimicrobial resistance has posed a significant challenge to healthcare systems worldwide, necessitating the exploration of novel antimicrobial agents and delivery systems. Among these, emulgels have garnered attention as versatile platforms for drug delivery due to their ability to combine the benefits of both emulsions and gels. Emulgels offer advantages such as enhanced stability, prolonged release of active ingredients, and improved patient compliance.

Flaxseed oil and thyme seed oil have been recognized for their antimicrobial properties attributed to their rich composition of bioactive compounds. Flaxseed oil contains α-linolenic acid, an omega-3 fatty acid, that exhibits antimicrobial activity against various pathogens. Thyme seed oil, on the other hand, is abundant in thymol, carvacrol, and other phenolic compounds known for their potent antimicrobial effects. The formulation and evaluation of an antimicrobial emulgel incorporating flaxseed and thyme seed oil present an innovative approach to combatting microbial infections. By harnessing the synergistic antimicrobial effects of these natural oils, the emulgel can offer a promising therapeutic option for various dermatological infections, including bacterial and fungal skin conditions.

This research holds the potential for addressing the growing challenge of antimicrobial resistance while leveraging the therapeutic benefits of natural compounds.The prevalence of antimicrobial resistance (AMR) is a global concern, affecting healthcare systems across the world, including India. The formulation and evaluation of antimicrobial emulgels utilizing flaxseed and thyme seed oil present a significant opportunity to address this challenge by offering alternative therapeutic options for various microbial infections.

Prevalence Worldwide: A significant majority of the global population still places high importance on traditional treatments for treating skin problems. There has been a renewed interest in the utilisation of medicinal plants in developing countries in recent years. The reason for this is because herbal medications are perceived as being safe and having fewer adverse effects in comparison to synthetic treatments. The use of herbal therapies topically has attracted considerable attention due to their extensive use and the poor comprehension of their benefit-risk ratio. Different medicinal plants are used to treat skin conditions and have antibacterial qualities. Gels, when applied topically to affected areas, exhibit superior efficacy compared to creams and ointments due to their ability to expedite medication delivery to the site of action. Neem (Azadirachta indica) and black tulsi (Ocimumsactum) are rich sources of beneficial phytochemicals, including flavonoids, anthraquinone glycosides, tannins, triterpenes, and steroids.

Current treatment approach: Emulgels and other semisolid drug forms have gained significant attention from scientists and business researchers in recent years. The skin plays a crucial role in the administration of both systemic and local medicines. While transdermal medicine delivery is generally convenient, it is important to note that not all drugs are effective when administered through the skin. Topical medicines are available in a wide range of forms, ranging from basic creams and solutions to advanced treatments utilising nanotechnology. Topical drug delivery methods are expected to gain popularity in the future, aiding patients in adhering to their treatments.Gel is a widely used and convenient method for administering medicine, effectively delivering the active ingredients to their targeted areas. The cross-linked and three-dimensional structure of gel enables it to effectively retain minuscule drug particles and facilitate their gradual release.A complex network of biomolecules has the ability to capture multiple fluid molecules within its three-dimensional structure.Due to their adhesive properties, gels have the ability to prolong the presence of medications on the skin. Many times, medicine creams are formulated by combining hydrophilic polymers with an appropriate amount of water. When polymers that have an

affinity for water dissolve, they transform into colloids that have a strong attraction to solvents. Due to the unique nature of their bodies, they undergo a transformation into a specific form of colloids known as self-associates.There are two types of self-association: those that can be reversed and those that are irreversible. Once created, the lyophilic gels are categorised into two groups: type 1 gels and type 2 gels. Type 1 gels, also known as hydrogels, consist of polymer chains that connect to one another at the cross-link level. One can appreciate the numerous advantages of gels, including their user-friendly nature, lack of coloration, non-greasy texture, and thixotropic properties. Unfortunately, there is one significant issue with them.

Material and Methods:

Flaxseed oil purchased from Hampi pure essential flax seed oil and Thyme seed oil purchased from Naturoman essential thymus oil and left all chemical carbopol 934, PG-400, Liquid paraffin, methyl parben, propyl paraben, triethanolamine, were arranged from Himt college of pharmacy, greater Noida.

Methods

Collection, Processing and procurement of Plant:

Collection, Processing and procurement of Flax Seed Oil

Flax seed oil was sourced from reputable suppliers who provide documentation on the oil's origin, extraction methods, and quality assurance, and stored in a cool, dark place to maintain its quality and potency.

Collection, Processing and procurement of Thymus Seed oil

Thyme oil was sourced from reputable suppliers who provide documentation on the oil's origin, extraction methods, and quality assurance, and stored in a cool, dark place to maintain its quality and potency.

Organoleptic Evaluation of Plants:

Organoleptic Evaluation of Flax Seed Oil

Organoleptic evaluation refers to the assessment of the properties of a substance based on the senses, such as taste, smell, and appearance.

Organoleptic Evaluation of Thymus Seed oil

Organoleptic evaluation refers to the assessment of the properties of a substance based on the senses, such as taste, smell, and appearance.

Methods for Characterization of Flaxseed Oil and Thyme Seed Oil:

Organoleptic Evaluation

For both flaxseed oil and thyme seed oil, start with an organoleptic evaluation. Observe the color and clarity of the oils, noting that flaxseed oil typically ranges from golden yellow to amber, while thyme seed oil usually varies from pale yellow to reddish-brown. Smell each oil to assess their characteristic aromas—flaxseed oil has a nutty aroma, whereas thyme seed oil possesses a strong herbal and spicy scent. Taste a small amount of each oil to ensure they have the expected flavors: a mild, slightly nutty flavor for flaxseed oil and a strong, pungent flavor for thyme seed oil.

Physicochemical Properties

Determine the physicochemical properties of both oils using the following methods:

Acid Value:

Weigh a known quantity of oil and dissolve it in an appropriate solvent (e.g., ethanol).

Titrate with a standard alkali solution (e.g., 0.1 N NaOH) using phenolphthalein as an indicator until a persistent pink color is observed.
Calculate the acid value, which indicates the amount of free fatty acids present in the oil.

Peroxide Value:

Mix a known quantity of oil with acetic acid and chloroform, then add potassium iodide solution.
Titrate the liberated iodine with sodium thiosulfate solution using starch as an indicator until the blue color disappears.
Calculate the peroxide value, which measures the extent of primary oxidation products (peroxides) in the oil.

Procedure:

Weigh a known quantity of oil (e.g., 1 g).
Dissolve it in ethanol.
Titrate with 0.1 N NaOH using phenolphthalein until a persistent pink color is observed.
Calculate the acid value.

Iodine Value:

Use the Wijs method by reacting the oil with Wijs reagent (iodine monochloride solution) and allowing it to react in the dark.
Add potassium iodide solution and titrate the liberated iodine with sodium thiosulfate solution using starch as an indicator.
Calculate the iodine value, which indicates the degree of unsaturation in the oil.

Saponification Value:

Saponify a known quantity of oil with a standardized potassium hydroxide solution under reflux.
Titrate the excess alkali with a standard acid solution (e.g., hydrochloric acid) using phenolphthalein as an indicator.
Calculate the saponification value, which measures the amount of alkali required to saponify a given quantity of oil.

Refractive Index:

Measure the refractive index of the oil at 20°C using a refractometer, which helps to identify and confirm the purity of the oil.

Specific Gravity:

Determine the specific gravity of the oil at 25°C using a pycnometer, which is the ratio of the density of the oil to the density of water.

Formulation of Herbal Emulgel:

The formulation of the antimicrobial emulgel using flaxseed oil and thymus seed oil involves the combination of various ingredients to create a stable, effective, and safe product. The process is designed to blend natural oils with other ingredients that contribute to the emulgel's texture, stability, and antimicrobial properties. Here is an overview of the formulation process:

Selection of Ingredients:

Thymus Seed Oil: Known for its antimicrobial properties, this oil will act as one of the active components in the emulgel.
Flaxseed Oil: Rich in omega-3 fatty acids, flaxseed oil contributes to the overall efficacy and nourishing properties of the emulgel.
Carbopol 934: A gelling agent used to create the desired viscosity and consistency for the emulgel base.
PG-400 (Propylene Glycol): Serves as a humectant and stabilizer, helping to maintain moisture and consistency in the emulgel.

Liquid Paraffin: Acts as a lubricant and moisturizer, contributing to the smooth application of the emulgel.
Methyl Paraben and Propyl Paraben: Used as preservatives to enhance the shelf-life and stability of the emulgel.
Triethanolamine: Utilized as a neutralizing agent to adjust the pH of the emulgel to an appropriate level for skin application.
Water: Forms the base of the emulgel and helps in the mixing and blending of ingredients.

Preparation of Emulgel:
Emulsion Formation: The oils (flaxseed oil and thymus seed oil) are emulsified with water using appropriate emulsifiers and surfactants to create a stable base.
Addition of Gelling Agent: Carbopol 934 is hydrated and dispersed in water to form a gel base for the emulgel.
Combination of Phases: The emulsified oil phase and gel base are blended together to achieve a homogenous mixture.
Adjustment of Consistency: Triethanolamine is added to adjust the pH of the emulgel, ensuring skin compatibility.
Addition of Preservatives: Methyl paraben and propyl paraben are added to the formulation to preserve the emulgel and extend its shelf-life.
Final Blending and Homogenization: All components are thoroughly mixed and homogenized to produce a uniform emulgel.
Here are three formulation batches for the antimicrobial emulgel using flaxseed oil and thymus seed oil, presented in a tabular form. The table includes the ingredient names and their respective concentrations for each batch.

Table no.1 composition of antimicrobial emulgel

Ingredients	Batch 1 (g)	Batch 2 (g)	Batch 3 (g)
Thymus Seed Oil	2	3	4
Flaxseed Oil	2	3	4
Carbopol 934	0.5	0.6	0.7
PG-400	5	6	7
Liquid Paraffin	3	3	3
Methyl Paraben	0.2	0.2	0.2
Propyl Paraben	0.1	0.1	0.1
Water	Q.S. to 100	Q.S. to 100	Q.S. to 100

Note: Q.S. stands for "quantum satis," which means "as much as needed" to make up the total weight of 100 grams for each batch.

Explanation of Batches:
Thymus Seed Oil and **Flaxseed Oil** concentrations vary across the three batches to explore the impact of different levels of these active ingredients on antimicrobial efficacy and overall formulation stability.
Carbopol 934 serves as the gelling agent and is adjusted slightly across the batches to test different levels of viscosity and consistency in the final product.
PG-400 (propylene glycol) concentrations are adjusted to maintain proper moisture and consistency in the emulgel.
Liquid Paraffin, **Methyl Paraben**, and **Propyl Paraben** concentrations remain constant across the batches to maintain the formulation's stability and preservation.
Triethanolamine is used to adjust the pH of the emulgel to ensure it is within a suitable range (6-7) for skin application.

Water is used to balance the formulation and make up the remaining percentage to achieve 100% of the batch.
These three batches can be tested and evaluated to determine the optimal formulation in terms of antimicrobial efficacy, stability, and overall performance.

Evaluation of Herbal Emulgel:
In the research project "Formulation and Evaluation of Antimicrobial Emulgel Using Flaxseed Oil and Thyme Seed Oil," the evaluation of the emulgel is a critical part of the study. The purpose of the evaluation is to assess the emulgel's physical, antimicrobial, and safety properties to ensure its efficacy and suitability for use.

Appearance test
The emulgel samples were observed for their visual characteristics, including color, texture, and homogeneity.
We noted any changes in appearance such as phase separation, consistency, or cloudiness over the storage period.
Any changes from the initial formulation were documented and analyzed.

Viscosity:
The viscosity of the emulgel was measured using a Brookfield viscometer.
The viscosity was calculated using the time measurement and the viscometer's constant.
The test was repeated multiple times for accuracy, and the average viscosity was reported.

pH
The pH of the emulgel was determined using a calibrated digital pH meter.
A sample of emulgel was taken and diluted with distilled water in a ratio (e.g., 1:9).
The mixture was stirred thoroughly and allowed to settle.
The pH meter probe was inserted into the mixture, and the pH value was recorded.
The test was conducted in triplicate, and the average pH was reported.

Spreadability Test
The spreadability of the emulgel was assessed using the circle spread method.
A known quantity of emulgel was placed on a glass plate and covered with another plate.
A weight was placed on the upper plate to apply a uniform pressure.
After a specified time (e.g., one minute), the diameter of the emulgel spread was measured.
The spreadability was calculated as the mean diameter of the emulgel spread.
Spreadability was calculated by using the formula,
$S=M.L/T$
Where,
S=Spreadability
M=weight tied to upper slide
L=length of glass slide
T=time taken to separate the slides completely from each other,
It was measured in terms of g.cm/sec

Extrudability Test
The extrudability of the emulgel was assessed using a collapsible tube method.
A filled tube of emulgel was placed between two plates and subjected to a constant weight.
The amount of emulgel extruded from the tube's nozzle was collected and measured over a specified time.
The extrudability was calculated as the weight of the emulgel extruded per unit time.
The process was repeated for multiple samples to ensure consistency.
Extrudability=Applied weight to extudeemulgel from tube (in g)/Area(in cm2)

Antimicrobial Study of Emulgel

The antimicrobial activity of the emulgel was evaluated using the agar well diffusion method. Agar plates were prepared and inoculated with the target microorganisms (e.g., bacteria and fungi).

Wells were created in the agar using a sterile cork borer, and a known quantity of emulgel was added to each well.

The plates were incubated at the appropriate temperature (e.g., 37°C for bacteria) for 24 hours.

After incubation, the zone of inhibition around each well was measured to assess the emulgel's antimicrobial efficacy.

The results were compared to control samples and standard antimicrobial agents to determine the emulgel's relative efficacy.

Result and Discussion:

Formulation of herbal Emulgel

The formulation of the antimicrobial emulgel using flaxseed oil and thymus seed oil involves the precise combination of various ingredients to create a stable, effective, and safe product. This process is designed to blend natural oils with other components that contribute to the emulgel's texture, stability, and antimicrobial properties. Below are the detailed steps and ingredients used in the formulation.

Preparation of emulgel

Evaluation of Herbal Emulgel:

Herbal Emulgel has been evaluated for the further uses. The result of evaluation Parameter are as follows.

Appearance Test

Table no.2

Batch	Initial Color	Initial Texture	Initial Homogeneity	Color after Storage	Texture after Storage	Homogeneity after Storage
1	Light Yellow	Smooth	Homogeneous	Light Yellow	Slightly Lumpy	Phase Separation
2	Light Yellow	Smooth	Homogeneous	Light Yellow	Smooth	Homogeneous
3	Light Yellow	Smooth	Homogeneous	Light Yellow	Smooth	Homogeneous

Viscosity

The Viscosity of Herbal Emulgel was found to be between in range of **14200 ± 100 to 15100 ± 360.55.** Table and Graph are listed as below.

Table no.3

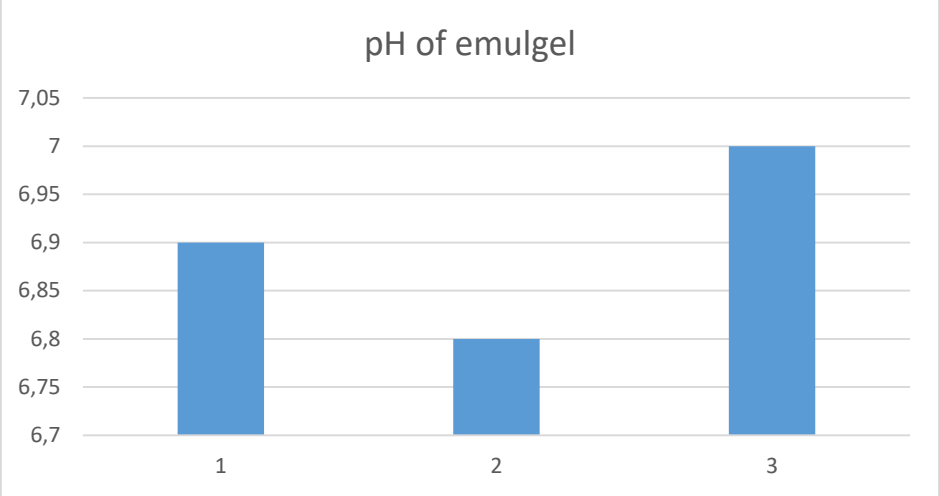
Batch	Trial 1	Trial 2	Trial 3	Average ±SD
Batch 1	14000	14300	14200	14200 ± 152.75
Batch 2	14500	14700	14600	14600 ±100
Batch 3	15000	15500	14800	15100 ±360.55

pH

The pH of Herbal Emulgel was found to be between in range of **6.8 ± 0.081 to 7 ± 0.047.** Table and Graph are listed as below.

Table no.4

Batch	pH - Trial 1	pH - Trial 2	pH - Trial 3	Average ±SD
1	6.9	7	6.8	6.9 ± 0.081
2	6.8	6.7	6.9	6.8 ± 0.081
3	7.0	7.1	7	7 ± 0.047



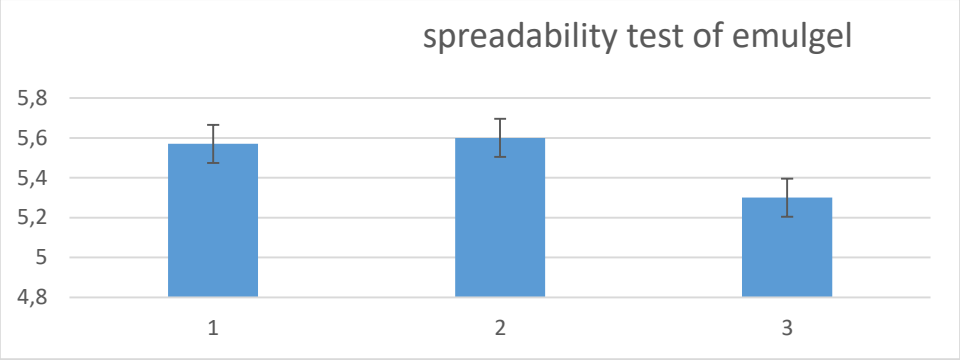
Spreadability Test

The Spreadability of Herbal Emulgel was found to be between in range of **5.30 ± 0.016 to 5.60 ± 0.016.** Table and Graph are listed as below.

Table no.5

Batch	Diameter (cm) – Trial 1	Diameter (cm) – Trial 2	Diameter (cm) – Trial 3	Average ±SD(cm)
1	5.8	5.7	5.9	**5.57 ± 0.339**
2	5.5	5.4	5.6	**5.60 ± 0.016**
3	5.2	5.3	5.1	**5.30 ± 0.016**

Table: Spreadability Test



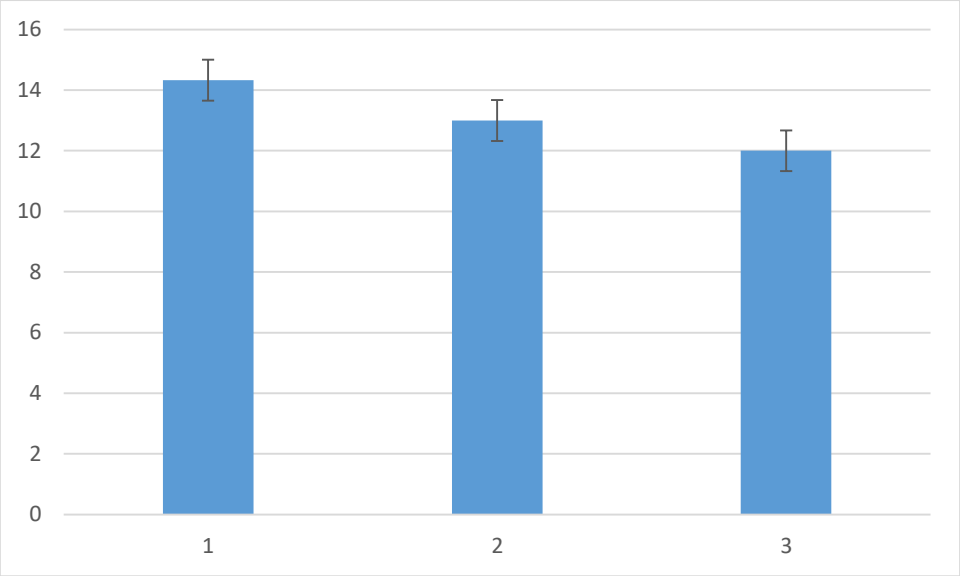
Extrudability Test

The Extrudability of herbalemulgel was found to be between in range 0f 13 ± 0.816 to 14.33± 0.471. table and graph are listed as below .

Table no.6

Batch	Extruded Amount (g) - Trial 1	Extruded Amount (g) - Trial 2	Extruded Amount (g) - Trial 3	Extrudability(%)	Remarks
1	14	15	14	14.33± 0.471	Good
2	13	12	14	14.33 ± 0.816	Excellent
3	12	11	13	13 ± 0.816	Low

Table: Extrudability Test



Schematically showing Extrudability of Batch 1, Batch 2, Batch 3

Antimicrobial Study of Emulgel

Standard range of antimicrobial of at different dose of ciprofloxacin and fluconazole (17 to 22 mm)

Table no.7

Batch	Concentration(mg/ml)	Zone of Inhibition (mm)–E.coli	Zone of Inhibition (mm) – S.	Zone of Inhibition (mm)– Pseudomonas

			Aureus	**aeruginosa**
1	**Ciprofloxacin**	**22.01**	**24.99**	**--**
2	**Fluconazole**	**--**	**--**	**20.56**
3	10mg	13.66	19.75	15.28
4	25mg	15.73	20.95	17.25
5	40mg	16.11	21.11	18.34

Table: Antimicrobial Study of Emulgel

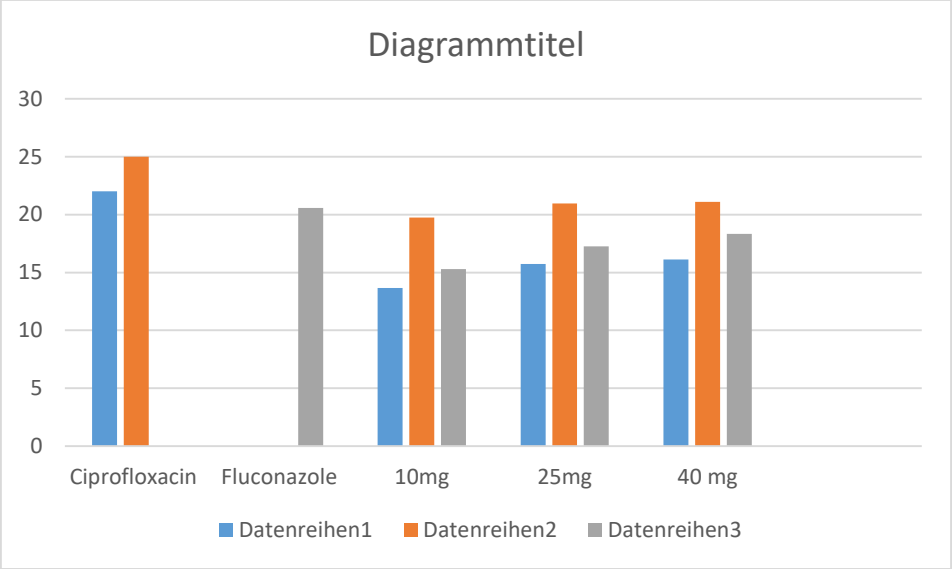


Figure: Comparison of Antimicrobial activity (Bacteria and Fungi) of Emulgel.

CONCULSION

All three batches of the herbal emulgel formulations demonstrated acceptable results across various parameters. However, Batch 2 displayed slightly superior performance in several key areas.Appearance: Remained smooth and homogeneous after storage.Viscosity: Consistent and stable viscosity.pH: Neutral pH (7.0), which is suitable for skin application.Spreadability: Optimal spreadability (5.60 cm), ensuring ease of application.Extrudability: Highest extruded amount (14 g), indicating better usability.Antimicrobial Activity: Highest zone of inhibition for both S. Aureus (21.11 mm) E.coli (16.11mm), Pseudomonas aneruginosa (18.34mm) indicating strong antimicrobial properties.

Given these observations, Batch 2 is the best candidate and can be used for further studies. Its superior performance in key areas makes it the most reliable and effective formulation for future pharmaceutical and cosmetic applications.

Conflict of Interest

The authors state no conflict of interest and have received no payment for this project.

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