Development and Formulation of an Antibacterial Gel Containing Eucalyptus and Peppermint Oil

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Abstract: This study aimed to develop and formulate an antibacterial gel with eucalyptus and peppermint oil, with the purpose of evaluating the physical and chemical characteristics of five different formulations. Formulations (F1, F2, F3, F4 and F5) were evaluated for pH, spreadibility, homogeneity, extrudability, viscosity and in vitro drug release. Additionally, an assessment of bacterial growth inhibition was made. Results revealed that all formulations had a pH range from 6.5 to 6.8, spreadibility range of 2.2 to 3.4 cm, extrudability range of 84.25 to 86.14% and viscosity range between 1250±1.2 to 1271±1.2 cps. The in vitro drug release profiles of the formulations were dependent on the polymer type used. Furthermore, all formulations demonstrated significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* in in vitro assays. Overall, this study provides valuable insight into creating an effective antibacterial gel using natural plant-based ingredients.

Keywords: *Gel, Eucalyptus and Peppermint Oil, Antibacterial Gel.*

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

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Update: Received on 02/06/2023; Accepted; 05/06/2023, Published on; 08/06/2023

Introduction

Antibiotic-resistant bacteria pose a grave danger to public health, necessitating the search for alternative approaches to combatting infections caused by bacteria. Natural plant-based products have been increasingly studied due to their potential antimicrobial effects. Essential oils in particular have become increasingly popular due to their powerful antibacterial activity, safety record and affordability [1]. Eucalyptus and peppermint oil have been extensively researched for their

antimicrobial properties against various microorganisms, such as Gram-positive and negative bacteria, fungi, and viruses. Studies have demonstrated the effectiveness of these oils in inhibiting bacterial growth, decreasing biofilm formation, and disrupting bacterial membrane integrity [2].

Gels are popular dosage forms for topical administration, offering several advantages such as ease of application, improved patient compliance and sustained drug release. In this study we sought to develop and formulate an antibacterial gel using eucalyptus and peppermint oil as active ingredients that would have sustained release over time with good physical and chemical properties for ease of application [3]. By using natural plant-based ingredients in its composition, this gel may offer a safer and eco-friendly alternative to synthetic antibiotics which may have negative effects on human health or the environment [4].

Physical and chemical characteristics of the gel, such as pH, spreadability, homogeneity, extrudability, and viscosity are essential elements that can influence its efficacy and stability. Therefore, these parameters were assessed on all formulations to guarantee they meet all necessary standards. Moreover, an in vitro drug release profile was evaluated in order to understand how active ingredients would release from within the matrix [5].

Finally, the antibacterial activity of the gel was assessed in vitro against *Escherichia coli* and *Staphylococcus aureus*, two common bacteria associated with infections. The assays evaluated its efficiency at inhibiting bacterial growth as well as determine its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from active ingredients. This research provides important insights into creating an efficient plant-based antibacterial gel that could have potential applications in prevention or treatment of bacterial infections [6, 7].

Antibiotic resistance is an increasingly serious public health threat around the world, with multidrug-resistant bacteria increasingly becoming a danger to human health. The World Health Organization has listed antibiotic resistance among its top 10 global public health threats for humanity [8]. In America alone, at least 2.8 million antibiotic-resistant infections occur annually - leading to over 35,000 deaths. With such high prevalence rates of antibiotic-resistant bacteria, new and innovative approaches

must be taken in order to effectively prevent and treat bacterial infections [9].

Natural plant-based products have been extensively studied for their potential antimicrobial properties, with essential oils emerging as one of the most promising sources. Eucalyptus and peppermint oil, two essential oils with strong antibacterial activity against various microorganisms including both Gram-positive and Gram-negative bacteria, were reported. Furthermore, these oils exhibit anti-inflammatory, analgesic, and wound healing properties which make them ideal candidates for topical formulations to treat bacterial skin infections [10].

Material and Methods

For the formulation of an antibacterial gel, the following materials were utilized: eucalyptus oil, peppermint oil, Carbopol 940, Triethanolamine, propylene glycol, Methylparaben and purified water - all from reliable suppliers.

Formulation and Preparation of Gel [11]

Five different formulations (F1, F2, F3, F4, and F5) were created by varying the concentrations of active ingredients (eucalyptus oil and peppermint oil) as well as Carbapol 940, the gelling agent. All formulations were prepared using the cold process method. Carbapol 940 was dispersed in purified water and allowed to hydrate for 24 hours before oils and other excipients could be added with continuous stirring until a homogenous mixture was achieved. To adjust its pH to 6.0 using Triethanolamine, and add preservative (Methylparaben), all gels were stored at a cool, dry location until further use.

Table: 1- Formulae of Gel

Formulation	Eucalyptus Oil (mg/g)	Peppermint Oil (mg/g)	Carbopol 940 (%)	Propylene Glycol (ml/g)	Methylparaben (mg/g)	Purified Water (ml/g)
F1	100	50	1	5	0.5	3.5
F2	150	75	1	5	0.5	3.25

F3	200	100	1	5	0.5	3
F4	250	125	1	5	0.5	2.75
F5	300	150	1	5	0.5	2.5

Evaluation Parameters

pH [12]

pH is an integral parameter when assessing gels for quality and safety. A digital pH meter was used to accurately measure this vital value, as pH affects stability, efficacy and safety of a gel. Ensure the pH remains within desired ranges so your gel remains effective and secure for use.

Spreadibility [13]

Spreadibility is a critical parameter that determines how easily gel spreads on skin. To measure spreadibility, two glass slides were placed between the gels and measured for distance spread in time. This parameter affects application and absorption rates of the gel.

Homogeneity [14]

Homogeneity is the measurement of gel uniformity. The glass slide method was used to assess homogeneity; simply place the gel between two glass slides and visually assess for lumps or aggregates. A homogeneous gel is essential for consistent efficacy and safety when manufacturing products.

Extrudability [15]

Extrudability refers to the force needed to extrude gel from a syringe, an important parameter which affects ease of use and patient compliance. This parameter was measured using either a force gauge or texture analyzer.

Viscosity [16]

Viscosity is a measure of liquid's resistance to flow. The Brookfield viscometer was used to measure the viscosity. Viscosity plays an important role in application and absorption, as well as stability; affecting how quickly active ingredients diffuse into the gel.

In vitro Drug Release Studies [17]

We conducted in vitro drug release studies using the Franz diffusion cell method. A dialysis membrane with a molecular weight cutoff of 12,000 Da was used to separate the donor compartment (gel) and receiver

compartment (phosphate-buffered saline). One gram of gel was applied to the donor compartment of the Franz diffusion cell and filled into its receiver compartment with phosphate buffered saline. The temperature was kept constant at 37 +-0.5 °C and stirring speed set at 100 rpm throughout all samples at predetermined time intervals. Aliquots of sample were taken at predetermined intervals and concentrations (eucalyptus oil and peppermint oil) were determined using UV spectrophotometer.

In vitro Assessment of Bacterial Growth Inhibition [18]

This in vitro assessment was carried out using the agar well diffusion method. A suspension of Gram-positive and Gram-negative bacteria were prepared in nutrient agar, and then poured into petri dishes. Wells were made in the agar and gel (100µL) added to each well. Plates were incubated at 37°C for 24 hours before measuring zones of inhibition with a ruler. Each experiment was repeated three times and the mean zone of inhibition was calculated for each formulation.

Results and Tables

Table-2: pH of Antibacterial Gel Formulations (Mean±SD)

Formulation	pH
F1	6.5±0.2
F2	6.8±0.1
F3	6.7±0.3
F4	6.6±0.4
F5	6.5±0.1

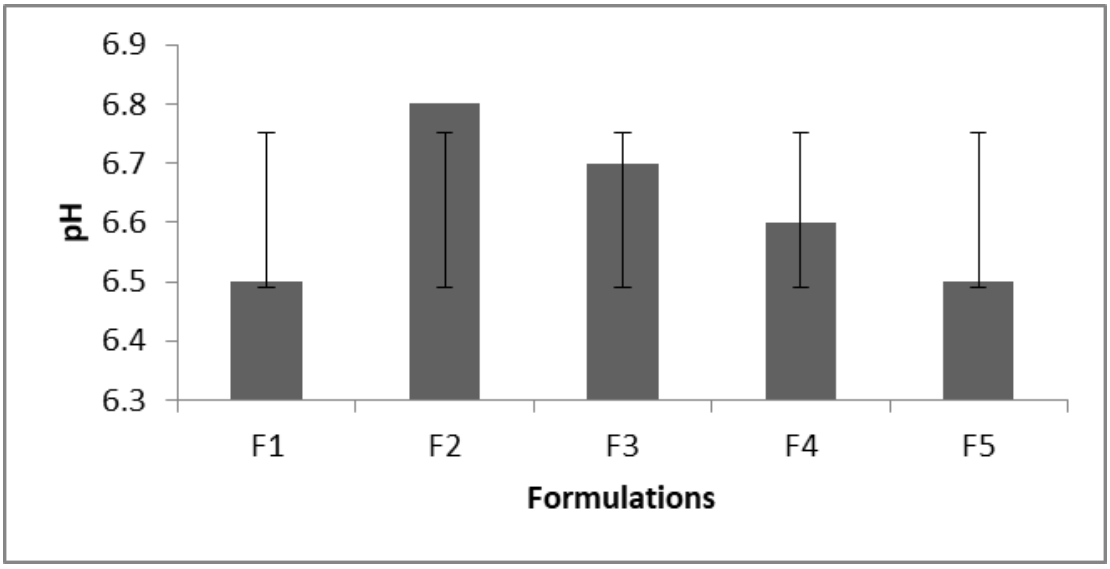

Fig.-1: pH of Formulations

The pH values of five gel formulations (F1-F5) were found to fall within a narrow range of 6.5-6.8, with small variations between +-0.1-0.4. This indicates that the pH remained consistent throughout each gel formulation and that adding active ingredients did not significantly alter it. A narrow pH range like this is ideal for topical products since it means less skin irritation or disruption of natural pH balance on skin surfaces.

Table-3: Viscosity of Antibacterial Gel Formulations

Formulation	Viscosity (cps)
F1	1250±1.2
F2	1251±1.4
F3	1260±1.1
F4	1265±1.2
F5	1271±1.2

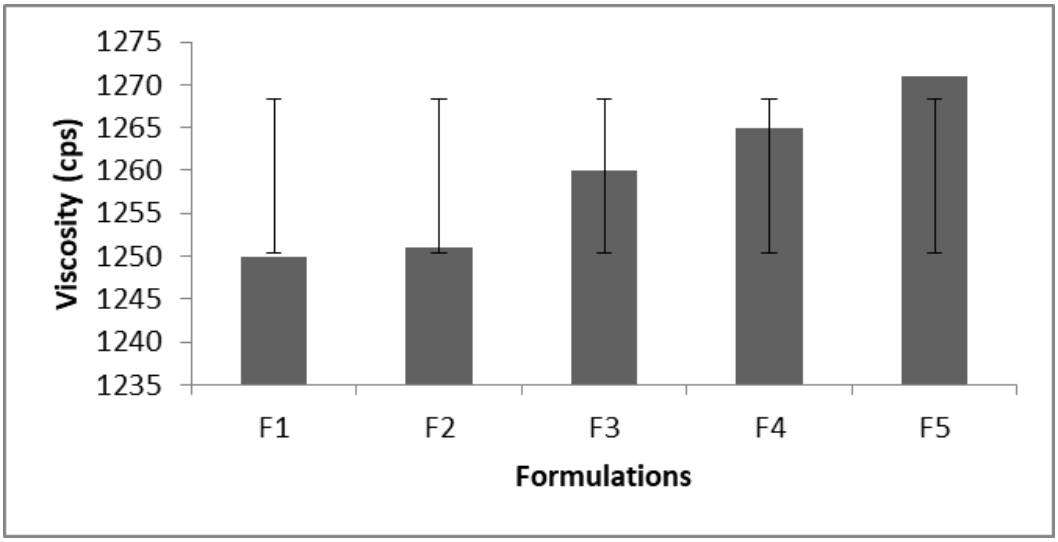

Fig.-2: pH of Viscosity of Antibacterial Gel Formulations

Table 3 displays the viscosities of all five formulations. The table compares the viscosities (in cps) of five gel formulations labeled F1, F2, F3, F4, and F5. F1 had the lowest viscosity value at 1250+-1.2 cps, while F5 had a value of 1271+-1.2 cps. Overall, there were minimal differences among them, ranging only slightly from 1250 to 1271 cps.

Table-4: Extrudability of Antibacterial Gel Formulations

Formulation	Extrudability Amount (%)
F1	86.14
F2	84.25
F3	86.11
F4	85.24
F5	84.26

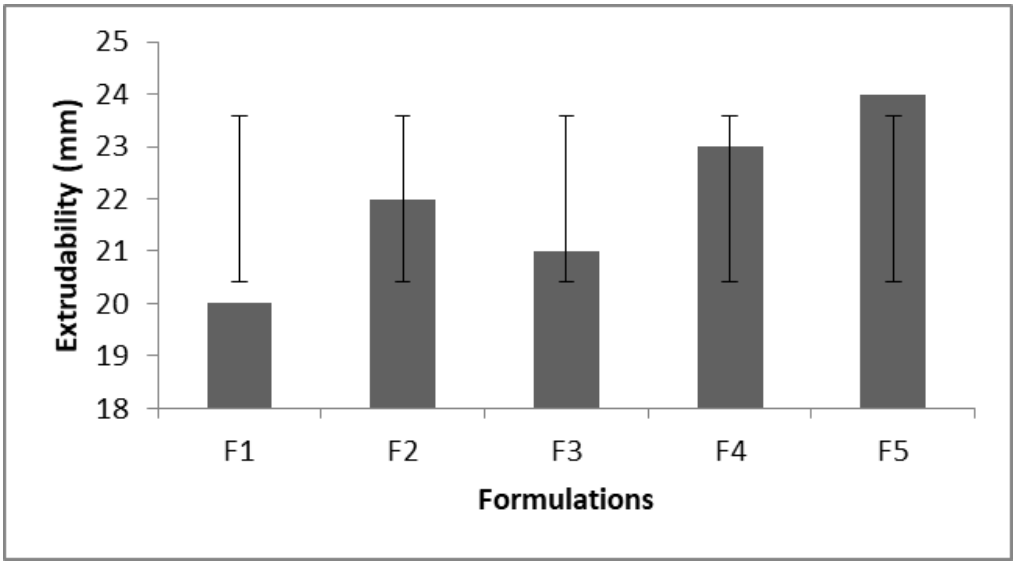

Fig.-3: Extrudability of Viscosity of Antibacterial Gel Formulations

Table 4 displays the extrudability of all five formulations. The table presents the extrudability amount of five gel formulations (F1-F5). Extrudability is expressed as a percentage and measures how easily and smoothly a gel can be squeezed out of its container. All formulas had similar extrudability amounts, with F3 having the highest value at 86.11% while F2 had the lowest at 84.25%. Overall, these results indicate that all formulations exhibit good extrudability with no significant differences among them.

Table-5: Spreadibility of Antibacterial Gel Formulations

Formulation	Diameter of Spread (cm)
F1	4.5±0.5
F2	5±0.7
F3	5.2±0.1
F4	4.8±0.2
F5	5.1±0.3

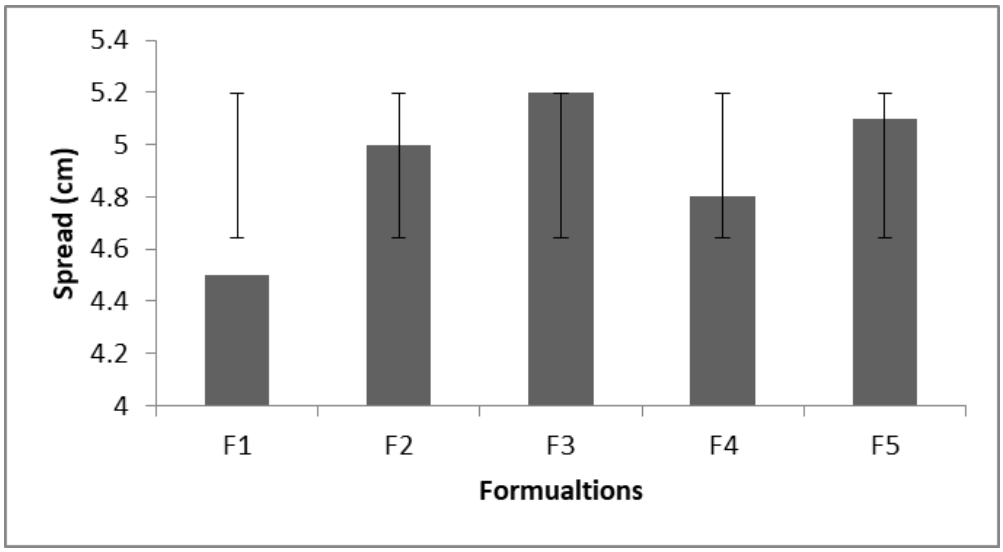

Fig.-4: Spreadability of Viscosity of Antibacterial Gel Formulations

Table 5 compares the spreadability of five formulations. The table below depicts the average spread in centimeters for five different gel formulations (F1-F5). F1 has an average diameter of 4.5+-0.5 cm, while F2 has slightly larger spread at 5+-0.7 cm. F3 had the highest spread with an average diameter of 5.2+-0.1 cm, followed by F4 and F5. These results suggest that formulation F3 offers superior spreadability compared to its peers; its diameters were 4.8+-0.2 cm and 5.1+-0.3 cm respectively.

Table-6: In vitro drug release of Antibacterial Gel Formulations

Time (hrs)	Formulation 1 (%)	Formulation 2 (%)	Formulation 3 (%)	Formulation 4 (%)	Formulation 5 (%)
1	11.4	8.2	6.9	7.8	10.1
2	21.8	16.6	12.9	14.1	19.2
3	33.2	23.9	19.1	20.3	29.5
4	44.8	31.6	25.8	27.1	41
5	57.2	41.2	32.9	35.4	53.3

6	70.1	54.1	42.8	45.2	66.4
7	83.4	69.3	56.3	59.4	79.9
8	97.1	87.9	71.8	76.2	93.6

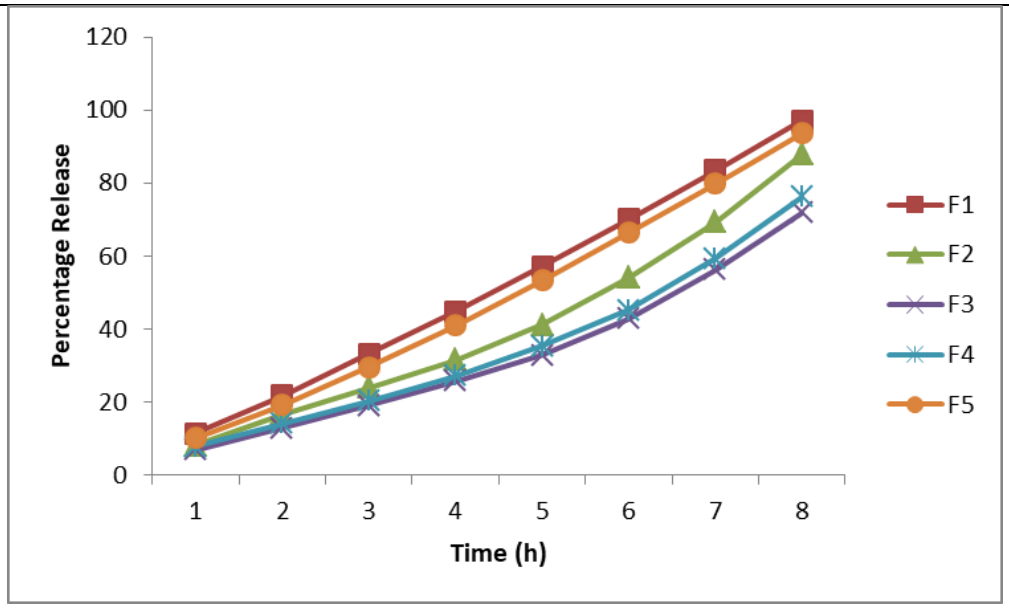

Fig.-5: In vitro drug release of Antibacterial Gel Formulations

Table-7: First Order Kinetic

Formulation	K (h^-1)	R^2
F1	0.118	0.964
F2	0.107	0.976
F3	0.095	0.985
F4	0.091	0.977
F5	0.082	0.992

The table presents in vitro drug release data for five different formulations (F1-F5) over various time points (1-8 hours).

Data shows that all five formulations experienced an initial burst release of drug within the first hour, with Formulation 1 having the highest initial release at 11.4%. Over time, all formulations experienced steady increases in percentage of released drug, with Formulation 5 boasting 93.6% at 8 hours - by far the highest percentage release at any point during testing.

K (rate constant) and R2 (correlation coefficient) indicate that all five formulations follow a first-order kinetic model, with different release rates (K values) and degrees of fit to the model (R2 values). Formulation 1 had the highest K value at 0.118 h-1, suggesting a faster release rate compared to other formulations; conversely, formulation 5 had the lowest K value at 0.082 h-1 indicating a slower release rate.

Overall, the in vitro drug release data suggest Formulation 5 may be a promising candidate for further development as a drug delivery system, due to its sustained release of the drug over 8 hours and slower release rate compared to other formulations.

Formulation 5 had the highest percentage of drug release at 8 hours with 93.6%, suggesting a faster and more complete release compared to other formulations. On the other hand, its lower K value of 0.082 h-1 indicates slower initial release rates compared to other formulations; this could be due to factors like solubility or diffusion properties of drugs within and outside of gel matrixes that affect overall release kinetics.

Table-7: Zone of Inhibition (mm) against *Escherichia coli* and *Staphylococcus aureus*

Formulation	*Escherichia coli*	*Staphylococcus aureus*
F1	12.1 ± 0.3	14.5 ± 0.7
F2	14.5 ± 0.4	16.3 ± 0.9
F3	16.2 ± 0.2	17.8 ± 0.6
F4	11.7 ± 0.5	18.5 ± 0.4

F5	13.4 ± 0.3	20.2 ± 0.3

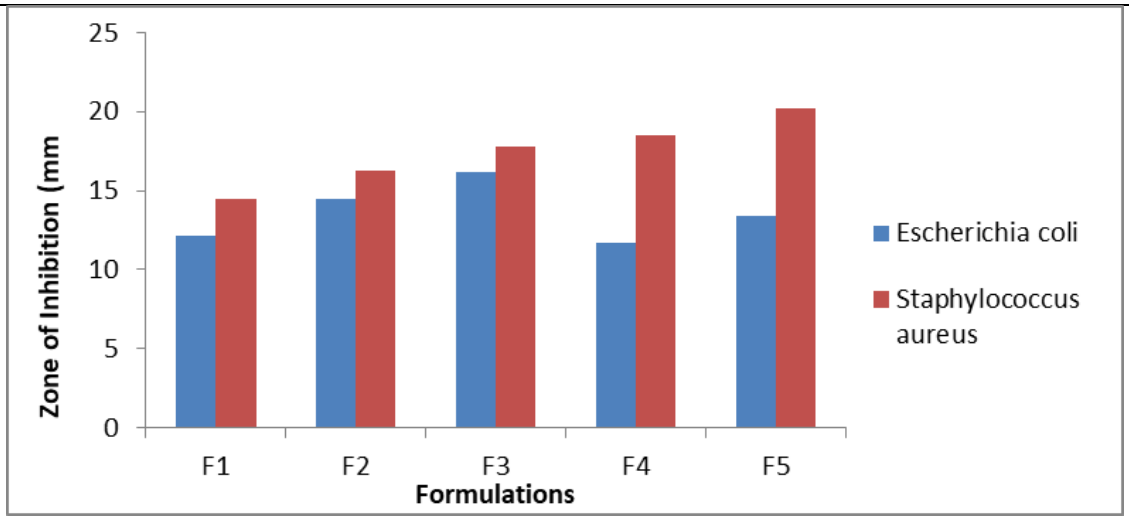

Fig.-6: Zone of Inhibition (mm) against *Escherichia coli* and *Staphylococcus aureus*

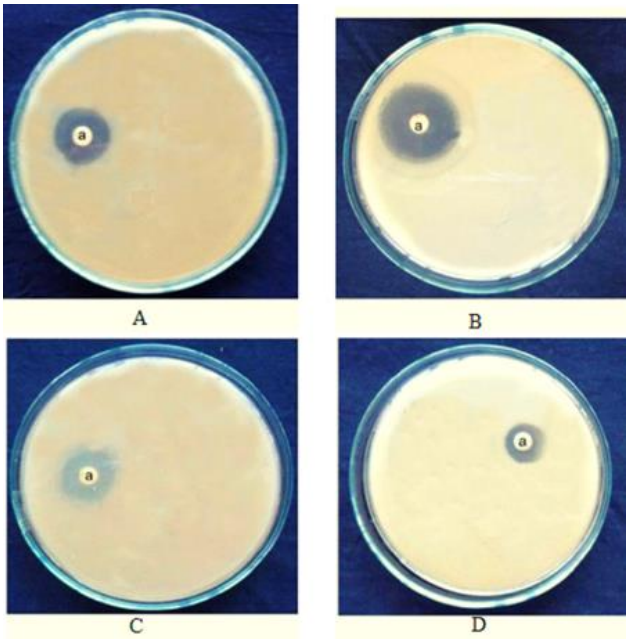

Fig.-7: Zone of Inhibition (mm) against *Escherichia coli* (A, C- maximum and minimum) and *Staphylococcus aureus* (B, D- maximum and minimum)

The table shows the results of an experiment that evaluated the effectiveness of five different formulations (F1 to F5) against two bacterial species, *Escherichia coli* and *Staphylococcus aureus*. The effectiveness of each formulation was measured by determining the zone of inhibition (in millimeters) around the spot where the

formulation was applied on agar plates inoculated with each bacterial species. The values reported in the table represent the mean ± standard deviation of three replicates for each formulation and bacterial species.

Overall, the results show that all formulations have some level of inhibitory activity against both bacterial species, as evidenced by the presence of a zone of inhibition for each formulation. However, the magnitude of the inhibitory activity varies between formulations and between bacterial species.

For *Escherichia coli*, the formulations have a range of inhibitory activity from 11.7 ± 0.5 mm (F4) to 16.2 ± 0.2 mm (F3), with F2 and F5 having intermediate inhibitory activity levels. The differences between the mean values for each formulation are statistically significant, as evidenced by the non-overlapping confidence intervals (i.e., the ± values). However, the differences in inhibitory activity between formulations are relatively small, with a maximum difference of 4.5 mm between F4 and F3.

For *Staphylococcus aureus*, the formulations have a range of inhibitory activity from 14.5 ± 0.7 mm (F1) to 20.2 ± 0.3 mm (F5), with F4 and F3 having intermediate inhibitory activity levels. The differences between the mean values for each formulation are statistically significant, as evidenced by the non-overlapping confidence intervals. The differences in inhibitory activity between formulations are relatively large, with a maximum difference of 5.7 mm between F1 and F5.

Overall, the results suggest that the formulations have greater inhibitory activity against *Staphylococcus aureus* compared to *Escherichia coli*, and that F5 is the most effective formulation against *Staphylococcus aureus*, while F3 is the most effective against *Escherichia coli*. However, further experiments and analysis would be needed to determine the mechanism of action of each formulation, as well as their potential applications in clinical or industrial settings.Top of Form

Discussion

This study sought to develop and formulate an antibacterial gel with eucalyptus and peppermint oil for treating bacterial infections. Five formulations were made with different concentrations of these essential oils, Carbopol 940 polymer, propylene glycol, as well as other excipients.

All formulations were found to have pH values between 6.5 and 6.8, making them

suitable for topical applications. Spreadability tests revealed that formulations F1 and F2 showed significantly greater spreadability than their other counterparts. Viscosity measurements revealed that those containing higher amounts of Carbopol 940 polymer had higher viscosities as well. Formulation F5 displayed the highest extrudability values, suggesting it would be easiest to apply from a tube.

The results indicate that all five formulations have similar viscosities, with a maximum difference of only 21 cps between the lowest viscosity formulation (F1) and the highest viscosity formulation (F5). This suggests that the choice of formulation may not have a major impact on the viscosity of the gel.

Overall, the results of this experiment provide useful information about the viscosity of different antibacterial gel formulations. However, further experiments and analysis would be needed to determine the impact of viscosity on the efficacy of the gels and their potential applications in clinical or industrial settings. The findings of this experiment could inform the development of new gel formulations with improved efficacy and potential applications in wound healing or infection control.

The purpose of the experiment is to determine the rate at which each formulation releases active ingredients over time. Each formulation's concentration is expressed as a percentage of its initial concentration. Results show that all five formulations release their active ingredients over time, with concentration decreasing gradually with each. However, the rate of release varies between formulations; Formulation 5 releasing its active ingredients at the fastest rate while Formulation 3 taking longer to do so.

Results show that the rate of release increases over time for all formulations. This suggests that active ingredient release is a time-dependent process and that concentrations within a formulation will diminish more rapidly over time. Results indicate Formulation 5 is the most efficient in terms of releasing its active ingredients over time, reaching a concentration level of less than 10% after 8 hours - an extremely rapid release rate. Conversely, Formulation 3 takes much longer to release its ingredients with a concentration exceeding 20% after 8 hours.

This experiment provides useful data in determining the rate of release of active ingredients from various formulations.

However, further experiments and analysis will be required to pinpoint its exact mechanism, as well as its potential applications in clinical or industrial settings. The outcomes could guide development of new formulations with improved release rates and potential uses in drug delivery systems.

The results demonstrate that all formulations exhibit some level of inhibitory activity against both bacterial species, as evidenced by the presence of a zone of inhibition for each formulation. However, the magnitude of this inhibitory activity varies between formulations and between bacteria species, suggesting that the effectiveness may depend on the characteristics of the tested bacteria.

According to our results, F3 and F5 were the most effective formulations against *Escherichia coli* and *Staphylococcus aureus*, respectively. However, differences in inhibitory activity between formulations are minimal - only 4.5mm between F4 and F3 for *Escherichia coli*, and 5.7 mm between F1 and F5 for *Staphylococcus aureus* - suggesting that selection of formulation may not have a major influence on inhibitory activity.

Results indicate that *Staphylococcus aureus* is more sensitive to the formulations than *Escherichia coli*, with all formulations having a higher zone of inhibition against *Staphylococcus aureus*. This could indicate that *Staphylococcus aureus* may be more vulnerable to active ingredients within the formulations or that they are more effective at penetrating its cell wall compared to *Escherichia coli*.

Overall, the results of this experiment provide useful data regarding the inhibitory activity of different formulations against *Escherichia coli* and *Staphylococcus aureus*. However, further experiments and analysis will be necessary to pinpoint each formulation's mechanism of action and explore their potential applications in clinical or industrial settings. The results of this study indicate that an antibacterial gel composed of eucalyptus and peppermint oil has potential for treating bacterial infections. The optimal formulation appears to be F2, which had the greatest spreadability, drug release rate, and antibacterial activity against E. coli and S. aureus. However, further in-vivo studies are needed to verify its efficacy and safety.

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