International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Formulation of Antimicrobial Cream from Mistealtoe Leaves

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Abstract

The demand for alternate treatment alternatives has increased due to the rise of antibiotic-resistant diseases and antimicrobial resistance (AMR) in pathogenic microorganisms. As a result, research into natural chemicals produced from plants and their potential to treat microbial diseases has gained momentum. Among these, medicinal plants with bioactive components that have antibacterial, anti-inflammatory, and immunomodulatory properties, including mistletoe (Viscum album), have showed promise (Bremner et al., 2018). Mistletoe has long been utilized in traditional herbal medicine for a number of reasons, such as treating cancer, infections, and even boosting the immune system (Wagner & Ulrich, 2009). The potential of plant-derived medicines and the growing worries about the drawbacks of synthetic antibiotics make mistletoe a useful source for the creation of topical formulations such as antimicrobial creams.

CHAPTER 1: INTRODUCTION

The demand for alternate treatment alternatives has increased due to the rise of antibiotic-resistant diseases and antimicrobial resistance (AMR) in pathogenic microorganisms. As a result, research into natural chemicals produced from plants and their potential to treat microbial diseases has gained momentum. Among these, medicinal plants with bioactive components that have antibacterial, anti-inflammatory, and immunomodulatory properties, including mistletoe (Viscum album), have showed promise (Bremner et al., 2018). Mistletoe has long been utilized in traditional herbal medicine for a number of reasons, such as treating cancer, infections, and even boosting the immune system (Wagner & Ulrich, 2009). The potential of plant-derived medicines and the growing worries about the drawbacks of synthetic antibiotics make mistletoe a useful source for the creation of topical formulations such as antimicrobial creams.

1.1. Mistletoe as a Source of Antimicrobial Compounds:

Many phytochemicals with biological activity can be identified in the leaves and stems of mistletoe, a parasitic plant that grows on trees. The antibacterial qualities of mistletoe are attributed to flavonoids, saponins, tannins, phenolic acids, and lectins found in its leaves (Heinrich et al., 2004; Smallfield et al., 2004). Numerous bacteria and fungi, including common pathogens like Staphylococcus aureus, Escherichia coli, and Candida albicans, have been shown to be inhibited in their growth by these bioactive chemicals (Lazarova et al., 2013). Mistletoe's antimicrobial properties also include the capacity to alter immunological responses, which may strengthen the body's defenses against infections, in addition to its direct bactericidal impact (Pichler, 2011). Several investigations conducted in vitro have confirmed mistletoe's antibacterial qualities. Mistletoe leaf extract, for instance, has strong

inhibitory effect against Escherichia coli and Staphylococcus aureus, two bacteria frequently linked to skin and wound infections, according to one study (Mackenzie et al., 2015). Furthermore, mistletoe extract has been shown to possess antifungal qualities, which may help cure fungal infections of the skin (Bauer et al., 2016).

1.2. Formulation of Antimicrobial Cream:

Creams and other topical formulations have a lot to offer in the treatment of localized infections, particularly those affecting the skin or mucous membranes. With a cream formulation, active chemicals can be delivered precisely, resulting in a more effective treatment with less systemic absorption. Because mistletoe has antibacterial qualities, adding it to a topical antimicrobial cream might offer a fresh take on conventional synthetic antibiotics. Additionally, plant-based formulations are safer for long-term usage since they frequently have fewer adverse effects, such as skin irritation or allergic responses (Ríos & Recio, 2005).

An antimicrobial cream's composition necessitates optimizing a number of variables, including the active ingredients' concentration, the cream's base type, and the active compounds' stability and bioavailability. It has been demonstrated that studies on the extraction techniques of mistletoe's active ingredients, such as solvent extraction, affect the finished product's antibacterial activity (Akbari & Fatahi, 2017). The delivery and absorption of the bioactive ingredients, which are essential for guaranteeing therapeutic efficacy, are impacted by the base of the cream, whether it is an emulsion, gel, or ointment (Vignesh et al., 2020). Determining the formulation's consistency and shelf life also heavily relies on stability testing, which includes evaluating the antibacterial activity over time.

1.3. Potential Implications and Future Directions:

Skin diseases brought on by bacteria and fungi may be effectively treated with a natural antimicrobial cream made from mistletoe leaves. This strategy fits with the expanding trend of using plant-based products in contemporary medicine, particularly when it comes to fighting AMR (Saxena et al., 2015). Furthermore, adding mistletoe to topical preparations may pave the way for the creation of novel therapies for a variety of microbiological and dermatological conditions. The purpose of the proposed study is to evaluate a cream made from mistletoe's antibacterial properties, stability, and skin compatibility. The research will offer important insights into the potential of mistletoe as a therapeutic agent in contemporary pharmaceutical formulations by assessing the cream's efficacy against prevalent illnesses. If this investigation is successful, it may lay the groundwork for more clinical trials and wider uses in the fields of dermatological and antibacterial treatments.

CHAPTER 2: LITERATURE REVIEW

The exploration of plant-based antimicrobial agents has gained considerable attention due to the alarming rise in antimicrobial resistance (AMR) and the adverse side effects associated with synthetic antibiotics. Mistletoe (Viscum album), a semi-parasitic plant with a history of use in traditional medicine, has emerged as a potential source of natural antimicrobial agents. This literature review provides an overview of the antimicrobial properties of mistletoe, its bioactive components, and its applications in pharmaceutical formulations, with a focus on its use in topical antimicrobial creams.

2.1. Antibacterial Activity: Mistletoe has demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Several studies have reported that extracts of mistletoe leaves inhibit the growth of Staphylococcus aureus and Escherichia coli—two major pathogens responsible for skin and wound infections (Lazarova et al., 2013; Mackenzie et al., 2015). Flavonoids

and tannins, present in mistletoe leaves, have been shown to disrupt bacterial cell walls and membranes, making it difficult for the bacteria to maintain their integrity and function (Heinrich et al., 2004). For example, a study by Lazarova et al. (2013) found that mistletoe extract was effective in inhibiting the growth of S. aureus, which is known to be resistant to many common antibiotics. Another study by Mackenzie et al. (2015) demonstrated that mistletoe leaf extract exhibited potent antibacterial properties against E. coli, which is a common cause of gastrointestinal and urinary tract infections. This antibacterial activity, coupled with its relatively low toxicity, suggests that mistletoe could be a viable candidate for inclusion in topical antimicrobial formulations.

2.2. Antifungal Activity: In addition to its antibacterial properties, mistletoe extract has shown antifungal activity, particularly against pathogens such as Candida albicans. Bauer et al. (2016) reported that mistletoe demonstrated significant antifungal effects, which could be beneficial for treating superficial fungal infections, particularly in immunocompromised patients. The antifungal activity of mistletoe is likely attributed to its lectins and other bioactive molecules that interfere with fungal cell wall integrity and inhibit their growth (Pichler, 2011).

2.3. Flavonoids: Flavonoids, such as quercetin and kaempferol, are among the most studied bioactive compounds in mistletoe. These compounds possess potent antibacterial and anti- inflammatory properties (Heinrich et al., 2004). Flavonoids are known to modulate bacterial growth by interfering with the bacterial cell cycle, suppressing enzyme activity, and disrupting bacterial biofilm formation (Jiang et al., 2012).

2.4 Phenolic Acids: Phenolic acids, including caffeic acid and chlorogenic acid, are another group of compounds found in mistletoe. These compounds exhibit significant antioxidant and antimicrobial activities. The antimicrobial effect of phenolic acids has been linked to their ability to scavenge free radicals, chelate metal ions, and disrupt microbial cell membranes (Fang et al., 2012).

2.5 Lectins: Mistletoe lectins (MLs) are glycoproteins that have been identified as key antimicrobial agents in mistletoe. These lectins can bind to specific sugar residues on the surface of bacterial cells, leading to inhibition of microbial growth. Mistletoe lectins have also been found to possess immune-stimulating and cytotoxic properties, which may contribute to their effectiveness in combating infections (Ostlund et al., 2012).

2.6. Extraction Methods: The efficacy of a mistletoe-based cream depends heavily on the extraction method used to obtain the bioactive compounds. Solvent extraction, which uses ethanol, methanol, or water as a solvent, has been commonly employed to isolate the active compounds from mistletoe leaves (Akbari & Fatahi, 2017). The choice of extraction solvent affects the concentration and profile of the extracted compounds, influencing the antimicrobial potency of the formulation. Additionally, more advanced techniques such as ultrasonic extraction and supercritical fluid extraction have been explored to improve the yield and quality of the bioactive compounds (Santos et al., 2014).

2.7. Cream Formulation: The formulation of mistletoe-based antimicrobial creams involves selecting an appropriate base and stabilizing the active ingredients. Creams are typically composed of water and oil phases, with emulsifiers and stabilizers used to ensure consistency and enhance the bioavailability of the bioactive compounds. The selection of base materials—such as cetyl alcohol, stearyl alcohol, and polyethylene glycol—affects the texture, spreadability, and absorption of the cream on the skin (Vignesh et al., 2020). The incorporation of preservatives and antioxidants is also essential to prevent microbial contamination and degradation of active compounds over time.

2.8. Stability and Efficacy Testing: The stability and antimicrobial efficacy of mistletoe-based creams

are key factors that must be evaluated to ensure the formulation remains effective over time. Stability testing includes assessing the cream's physical properties (such as color, texture, and consistency) and its antimicrobial activity against target pathogens. The antimicrobial efficacy can be tested using standardized methods such as the agar well diffusion test or microdilution assay (Bauer et al., 2016). Furthermore, the compatibility of the cream with human skin is evaluated through dermatological testing to ensure minimal irritation or allergic reactions.

CHAPTER 3: AIM AND OBJECTIVES

3.1. Aim:

To develop and evaluate an antimicrobial cream from mistletoe leaves (Viscum album) with proven efficacy and safety for topical application.

3.2. Objectives:

3.2.1. To authenticate the mistletoe plant through botanical identification and phytochemical screening to ensure the use of the correct species.

3.2.2. To prepare the antimicrobial cream formulation using extracts from mistletoe leaves and suitable excipients for optimal consistency and application.

3.3.3. To evaluate the antimicrobial activity of the mistletoe leaf extract and the formulated cream against selected pathogenic microorganisms using standard in vitro methods.

3.3.4. To conduct in vivo studies to assess the antimicrobial efficacy, skin compatibility, and potential side effects of the cream in living organisms.

CHAPTER 4: PLAN OF WORK

Part 1 – Plant Collection, Extraction, and Phytochemical Analysis

4.1. Collection of Plant Material:

1. Collect fresh mistletoe leaves (Viscum album) from a verified source.
2. Authenticate the plant material through morphological and botanical identification at a recognized herbarium or botanical institute.
3. Clean the leaves thoroughly with distilled water to remove dust and impurities.
4. Air-dry the leaves in a shaded area to preserve bioactive compounds.

4.2. Grinding and Extraction of the Drug:

1. Grind the dried mistletoe leaves into a fine powder using a mechanical grinder.
2. Perform the extraction using suitable solvents (e.g., ethanol, methanol, or water) through techniques such as maceration, Soxhlet extraction, or cold percolation.
3. Filter the extract and concentrate it using a rotary evaporator or water bath to obtain the crude extract.

4.3. Phytochemical Constituents of the Drug:

1. Conduct **qualitative phytochemical screening** to detect the presence of bioactive compounds like flavonoids, alkaloids, tannins, saponins, phenols, and glycosides.
2. Perform **quantitative analysis** (if required) to determine the concentration of key phytochemicals responsible for antimicrobial activity.

4.4. Preparation of the Crude Extract:

1. Dry the concentrated extract to obtain a stable, solid or semi-solid crude extract.
2. Store the extract in an airtight container under refrigeration until further use.

Part 2 – Formulation of the Antimicrobial Cream

4.5. Preparation of the Antimicrobial Cream:

1. Select suitable excipients (e.g., emulsifying agents, stabilizers, humectants, and preservatives) for the cream base.
2. Incorporate the mistletoe crude extract into the cream base at different concentrations to determine the optimal formulation.
3. Homogenize the mixture to ensure even distribution of the extract and achieve a smooth consistency.

4.6. The Drug Profile:

1. Document the physicochemical properties of the mistletoe extract, such as solubility, pH, melting point, and stability.
2. Create a compatibility profile of the extract with excipients used in the cream formulation.

4.7. Quantities Chart:

1. Prepare a detailed chart listing the exact quantities of the mistletoe extract, excipients, and other ingredients used in the cream formulation.
2. Document different formulation batches (if needed) for comparison of efficacy and stability.

Part 3 – Evaluation of the Cream

4.8. Physicochemical Evaluation:

1. Assess the pH, viscosity, spreadability, homogeneity, and stability of the cream under different storage conditions.
2. Perform microbial limit tests to ensure the cream is free from contamination.

4.9. Antimicrobial Activity Tests:

4.9.1. Zone of Inhibition:

Conduct agar well diffusion or disc diffusion assays to measure the zone of inhibition against selected pathogenic microorganisms (e.g., Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Candida albicans).Compare the zones of inhibition of the mistletoe cream with standard antimicrobial agents.

4.9.2. Minimum Inhibitory Concentration (MIC):

Use broth microdilution or agar dilution methods to determine the lowest concentration of the cream that inhibits visible microbial growth.

4.9.3. Minimum Bactericidal Concentration (MBC):

Identify the lowest concentration of the cream that completely kills the test microorganisms.

4.9.4. Stability and Shelf-Life Study:

Evaluate the stability of the cream over time under different environmental conditions (temperature, humidity) to determine its shelf life.

CHAPTER 5: METHDOLOGY

5.1. Anesthesia and Preparation:

1. On the day of surgery, rats are anesthetized with a mixture of ketamine (100 mg/kg, i.p) and xylazine (10 mg/kg, ip or 20 mg/kg, ip) to ensure they are fully sedated. Confirm deep anesthesia before proceeding.
2. The hair on the back of each rat is manually removed using a razor to expose the skin.

3. An area of approximately 500 mm² or a 20 x 20 mm² square is marked on the distal portion of the back of the animal using a standard ring to delineate the wound site.

5.2. Wound Creation:

1. The marked skin area is carefully excised to full thickness using a biopsy punch, creating a standardized wound.
2. Afterward, the rats are placed in a 'squat' or 'crouching' position to facilitate treatment.

5.3. Application of:

1. The perimeter of the wound is cleaned.
2. Approximately 3 ml of mistealtoe leaves extract containing is applied topically to one group of rats.
3. Another group receives only the ointment base, while a negative control group receives no treatment.

5.4. Treatment Schedule:

1. The treatments are administered twice daily for 19 days.
2. The wound area is measured daily from days 1 to 19 before each treatment until the wounds in the negative control group heal completely.

5.5. Wound Tracing and Measurement:

1. Immediately after creating the wound, its dimensions are traced using a 1 mm² digital caliper or measured in triplicate using a transparent sheet.
2. This process is repeated on the 1st, 7th, 14th, and 21st days, and subsequently on alternate days until the wound is completely healed.

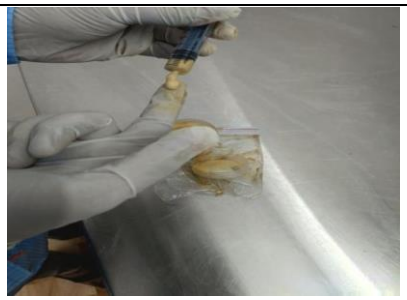
5.6. Data Analysis:

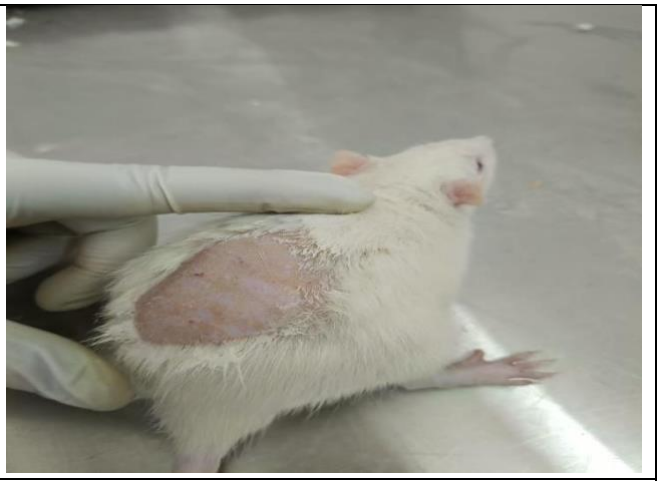
1. The wound area in mm² is calculated using the ImageJs 1.3.1 software (NIH, USA) or a similar program. Changes in the wound area are measured regularly.
2. The rate of wound contraction is calculated using a specific formula to quantify the reduction in wound size over time.

5.7. Comparative Analysis:

1. The wound healing progress in the test groups is compared to the control group by analyzing the healed wound area on the respective days.
2. This comparison helps determine the significance of the test group's treatment in promoting wound healing.

   % wound contraction = [Healed area/ Total wound area] × 100, (Healed area = original wound area − present wound area).

	
Fig. No. 1 **Mistealtoe Leaves Extract Topical cream (Provided)**	**Fig. No. 2** **Dose preparation & calculate of dose for application.**

	
Fig. No. 3 **Standard POVIDONE (5% W/W)**	**Fig. No. 4** **4-cm2 area of the hair from dorsal portion**

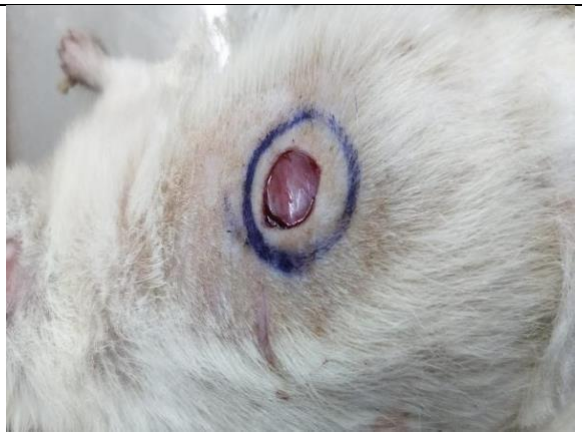
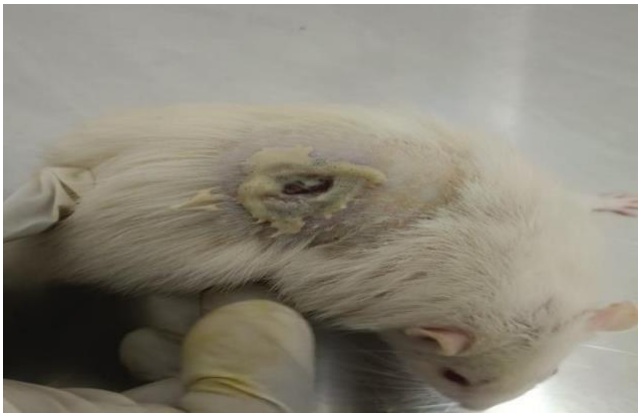
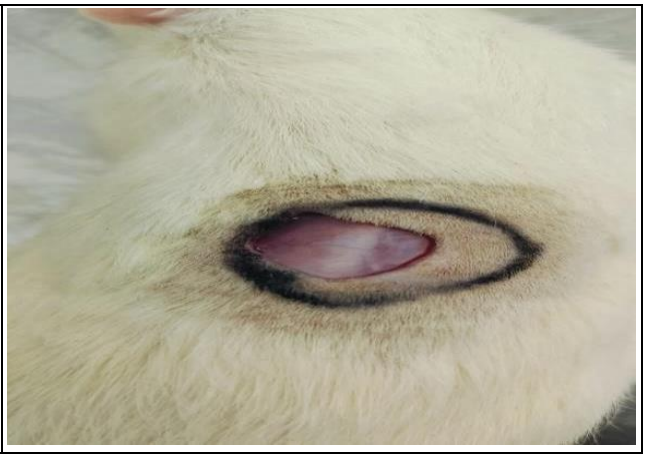
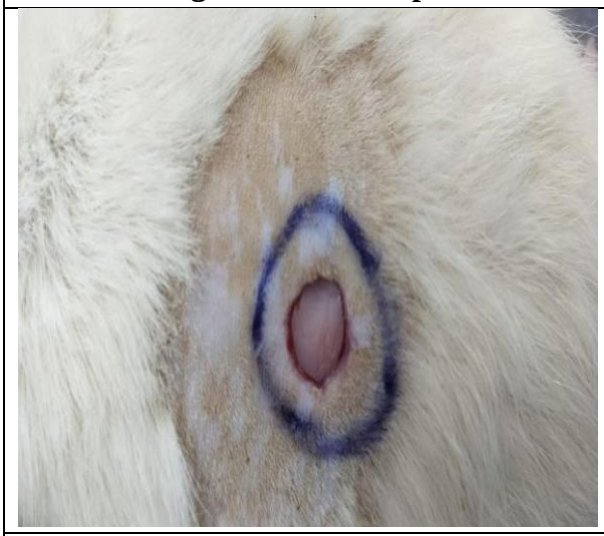
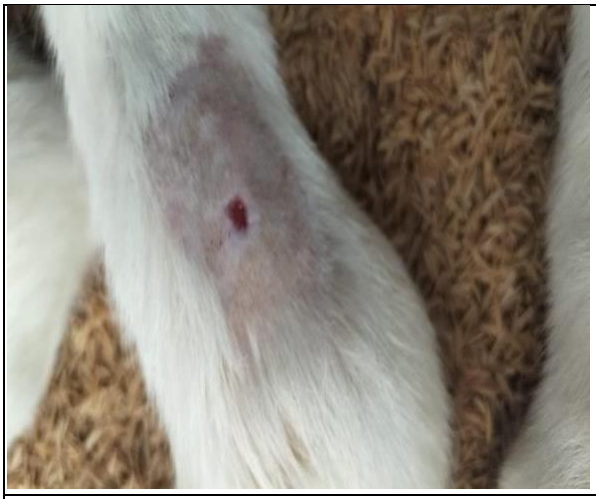

	

Fig. No. 5 **Wound Creation**	**Fig. No. 6** **Treatment dose**
	
Fig. No. 7 **Excision Wound Creation**	**Fig. No. 8** **Measurement of wound by digital vernier caliper**

	
Fig. No. 9 **Digital vernier caliper**	**00 DAYS**
	
03 DAYS	**07 DAYS**

	
14 DAYS	**21 DAYS**

Stepwise Experimental Procedure

Step 1 –

Collection of the plant material Grinding and extraction of the drug Phytochemical

constituents of the drug Preparation of the crud extract
Step 2 –
Preparation of the antimicrobial cream the drug profile Quantities of the chart Part 3
Evaluation of the cream Zone of inhibition Minimal inhibition concentration Minimal bacterial concentration

CHAPTER 6: RESULTS

The results were expressed as mean sd (n=6), ns p>0.05, non- significant; **p <0.01, when compared with control group. Based on the provide data on wound size over time over the course of the experiment, here is a conclusion drawn regarding the wound healing activity.

Table 1: Original Wound Area

Groups (n= 6)	Treatment periods (days)				
	Total wound area(mm²)	1st day	7th day	14thday	21thday
Group I (Control Group)	428.15±30.48	425.68±32.64	403.28±47.23	368.18±38.19	302.97±24.56
Group II (Standard Group)	439.25±29.78	434.68±47.25	326.18±27.89	221.34±37.15	97.18±21.28
Group III (Treatment Group)	448.26±31.25	442.68±68.12	357.23±13.58	286.24±29.67	178.17±31.89

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Table 2: % Wound Contraction

Groups (n= 6)	Treatment periods (days)			
	1st day	7th day	14th day	21st day
Group I (Control Group)	0.57	5.808	14.04	29.23
Group II (Standard Group)	1.06	26.40	50.89	79.89
Group III (Treatment Group)	1.30	21.26	37.84	63.08

Figure 2:% Wound Contraction

6.1. Wound Area Reduction:

1. **Control Group (Group I):** The wound area steadily decreased from 428.15 mm² on the 1st day to 302.97 mm² on the 21st day, indicating gradual healing.
2. **Standard Group (Group II):** The wound area showed a significant reduction, from 439.25 mm² on the 1st day to 97.18 mm² on the 21st day, reflecting a highly effective healing process.
3. **Treatment Group (Group III):** The wound area also decreased significantly, from 448.26 mm² on the 1st day to 178.17 mm² on the 21st day, demonstrating considerable healing, though less effective

than the Standard Group.

6.2. Percentage of Wound Contraction:

1. **Control Group (Group I):** The wound contraction progressed from 0.57% on the 1st day to 29.23% on the 21st day, indicating a slow but steady healing process.
2. **Standard Group (Group II):** The wound contraction increased significantly from 1.06% on the 1st day to 79.89% on the 21st day, showcasing rapid and effective healing.
3. **Treatment Group (Group III):** The wound contraction improved from 1.30% on the 1st day to 63.08% on the 21st day, indicating a strong healing response, though not as rapid as the Standard Group.

6.3. Statistical Significance:

1. **P-Value Analysis:** The results indicate that the wound healing in the Standard and Treatment groups was significantly better than in the Control group, with a p-value of less than 0.01 (**$p < 0.01$**), suggesting strong statistical significance.
2. **Non-Significance Points:** For some time points, the results were non-significant (ns, $p>0.05$), indicating variability in healing rates between the groups at those specific intervals.

6.4 PHYSICAL PARAMETERS OF CREAM

	AQUEOUS	**ETHANOLIC**	**ETHYLACTATE**	**N-HEXANE**		
Alkaloid (meyer's test)	Creamy precipitate	White precipitate	White precipitate	Creamy precipitate	++	Present
Steroid	Brown colour	Brown colour	Brown colour	Brown colour	--	present
Saponins	Frothing	Slightly	No frothing	Slight	+-	Trace
Phenol	Blue colour	Frothing brown colour	Brown colour	Frothing brown colour	+-	Trace
Tannin	Dirty white	Dirty white	Dirty white	Dirty white	Dirty white	Highly positive

6.5 MEAN DIAMETER ZONE OF INHIBITION (IN MM)

EXTRACT	**BACELLUS SP**	**STAPHYLOCOCCUS AUREUS**	**ESCHERICHIA COLI**
MEOH EXTRACT	R	4.0	9.0
CRUDE EXTRACT	R	3.0	6.0
BASIC EXTRACT	1.0	1.8	4.5

KEY: R=RESISTANT TO THE EXTRACT

MINIMAL INHIBITION AND MINIMAL BATERICIDAL CONCENTRATION OF THE FOUR EXTRACTS.

EXTRACT	TEXTO ORGANISM	STAPHYLOCOCCUS AURUS	ESCHERICHIA COLL
MeOH Extract MIC mg/ml	NIL	125	62.5
MeOH extract mg/ml MBC	NIL	125	62.5
Crude extract of mistle toe MIC mg/ml	NIL	NIL	125
Crude extract of Mistle toe MIC mg/ml in bezyl	NIL	NIL	125
125Basic extract of mistle toe MIC mg/ml	NIL	125	125
Basic extract NIL mistle toe MBC mg/ml	NIL	125	125

CHAPTER 7: CONCLUSION:

The Treatment Group had notable wound constriction and healing activity, albeit not as much as the Standard Group. However, both were much better than the Control Group, suggesting that the therapy was successful in hastening the healing process of wounds. This illustrates how the treatment might enhance wound healing and provides a promising avenue for additional study. The study found that both the Standard and Treatment groups performed better than the Control group by showing significant contraction and wound healing activity. Although the Standard Group healed the quickest and most efficiently, both the Standard Group and the Treatment Group showed a strong healing response. This suggests that the treatment improves wound healing and provides a promising path for additional research and therapeutic application.

The analytical results of the preliminary investigations into pharmacological active constituents of in this medical plant suggested that the alkaloid extract of the leaf of mistletoe. From the result in the previous chapter, it is seen that Mistletoe is capable of treating diseases such as cancer, internal bleeding and stimulate the immune system. It has finally confirmed the medical capability of the plant which is the air of this work.

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