ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul538

The Potential Wound Healing Activity of Mussaenda Philippica (Rubiaceae) Methanolic Leaf Extract

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Publication Date: 2025/07/21

Abstract: This study investigated the wound-healing efficacy of *Mussaenda philippica* leaf extract, a plant traditionally used for wound management. Methanol-extracted leaf compounds were formulated into topical ointments at 1%, 5%, and 10% concentrations and tested on excised wounds in male mice, with 10% povidone-iodine and plain ointment serving as positive and negative controls, respectively. Phytochemical analysis identified tannins, flavonoids, and saponins-bioactive compounds associated with healing mechanisms. Acute toxicity assessments demonstrated no adverse effects, confirming the extract's safety.

The 10% formulation exhibited the most potent wound-healing activity, achieving complete epithelialization within 14 days, comparable to the 10% povidone-iodine group. The 5% concentration also showed significant efficacy, outperforming the negative control, while the 1% formulation demonstrated minimal therapeutic impact. These findings validate *M. philippica's* traditional use and suggest its potential as a safe, cost-effective alternative to synthetic wound care agents. Further pharmacological and clinical studies are recommended to optimize dosage and evaluate translational applicability in humans.

Keywords: *Mussaenda Philippica; Wound Healing; Plant Extract; Mice; Traditional Medicine.*

How to Cite: Norhanifah C. Didato; Junnin Gay L. Garay; Vera Marie T. Gonesto; Patricia Mae B. Jarabe; Norjannah D. Palawan (2025). The Potential Wound Healing Activity of Mussaenda Philippica (Rubiaceae) Methanolic Leaf Extract. *International Journal of Innovative Science and Research Technology,* 10(7), 1382-1390. https://doi.org/10.38124/ijisrt/25jul538

I. INTRODUCTION

Wounds are one of the most common health issues today. They often heal slowly if not properly managed [6]. Effective and safe promoters of wound healing have been of great interest amongst scientists in order to approach the therapeutic potential of natural plant products. Among these are *Mussaenda philippica*, known as Doña Aurora, which had been used in the traditional treatment of various afflictions [6].

A closer look at the relevant literature served as a hypothesis that this isolate from *M. philippica* might have possessed a potential wound healing [8] and it exhibited partial antimicrobial and antioxidant effects; apart from its association with the traditional application of *M. philippica* for various types of wounds to heal [8]. Additional supporting evidence came from the overall composition of its phytochemical components present in such plant genus: flavonoids, tannins, saponins [6] [8]. Such compounds were found to possess anticatabolic, anti-inflammatory, antimicrobial, and tissue regenerative properties [13].

The wound healing activity of the methanolic leaf extract of *M. philippica* (Doña Aurora) was investigated in an in vivo excision wound model using Swiss albino male mice. This model was selected due to its well-defined genetic background, ease of handling, and reproducibility in wound healing studies [4] [15]. The study aimed to validate the traditional use of *M. philippica* in wound management by evaluating the extract's efficacy, with wound contraction rate as the primary parameter. Wound area reduction was calculated as a percentage of the original size measured on day zero. Through statistical analysis, the optimal concentration of the methanolic leaf extract demonstrating the highest wound contraction rate and lowest residual wound area was identified to provide evidence-based support for its therapeutic potential.

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II. MATERIALS AND METHODS

➢ *Plant Collection*

The plant *M. philippica* was collected from Bacolod, Poblacion, Zone 6 Lanao Del Norte 8.1880° N, 124.0224° E.

➢ *Plant Authentication*

The authentication process of the plant was carried out by a plant biologist at the Department of Biological Sciences, College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology.

➢ *Preparation of M. philippica*

Plant sample of two sacks were air dried under shade at room temperature (20˚C-25˚C). Dried leaves were pulverized using Electric Coffee Grinder and then sieved through a 40 and 60 mesh sieve to get fined powder and was packed in a clean plastic container.

➢ *Methanolic extraction of M. philippica pulverized leaves*

Percolator was used as it allowed the extractant to be maximally saturated with active substances as it passed through the series of percolators, thus maximizing yield and concentration of the desired bioactive compounds [11].

The dried and powdered leaves of *M. philippica* (100 g) were moistened with 80% methanol in a beaker. Subsequently, the moistened powdered leaves of *M. philippica* were transferred into the percolator with cotton and filter paper inside it, 400 ml of methanol was added to soak the leaves and was allowed to stand for 24 hours with the lower end closed. After 3 days the liquid was allowed to drip slowly; enough methanol was added repeatedly to maintain a layer of solvent above the pulverized leaves then plant extract was obtained. The obtained extract was then transferred to a beaker covered properly with a foil. The plant extract was then placed in a water bath (40 ˚C) and was allowed to evaporate until a thick crude extract was obtained [1].

Triplicated analysis of 100g samples were used to determine the average percentage yield of the crude methanolic leaf extract of *M. philippica* by using the formula given below

$$\% \text{ (w/w) yield} = \frac{\textit{wieght of crude extract (g)}}{\textit{weight of dried leaf sample (g)}} \times 100$$

➢ *Phytochemical Confirmatory Test*

- *Ferric Chloride Test for Tannins*

In a test tube, 5 ml of the extract was added, followed by 1 ml of water and 1 to 2 drops of ferric chloride solution ($FeCl_3$). A greenish-black color was observed [14].

- *Alkaline Reagent Tests for Flavonoids*

1 ml of the extract was placed into a test tube. Then, a few drops of sodium hydroxide solution were added. Shaken and produced deep yellow coloration which disappeared upon mixing with dilute acid. This indicates the presence of flavonoids in the extract [1].

- *Test for Saponins*

A drop of Na2CO3 solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins [7].

➢ *Acute Toxicity Testing*

- *OECD 402: Acute Dermal Toxicity*

The acute dermal toxicity study, as outlined in OECD Test Guideline 402, was carried out for establishing the hazardous properties of methanolic leaf extract of *M. philippica* by application on the skin. This experiment provided the data related to the health hazards expected to appear because of dermal exposure within a short-term time frame.

- *Selection of Animals*

Male mice were commonly used in wound healing studies due to their well-defined genetic background, ease of handling, and reproducibility of results [3]. Male mice were often preferred for wound healing studies over female mice because males tended to respond more similarly to interventions for wound healing. The response did not have as much variability due to hormonal fluctuation, which could happen in the female mice at some point in the estrous cycle [5]. Healthy young adult Swiss Albino male mice (8-10 weeks old and weighing 20-30g were used [9]. These were obtained from CDO Mice Breeder, Cagayan de Oro City, Philippines.

- *Housing and Feeding Condition*

The room was kept controlled at a temperature of 22°C ±3°C. The relative humidity in the room was maintained between 30% and 70% while keeping it between 50% and 60% except when the room is being cleaned. Artificial lighting with a 12-hour light cycle and 12-hour dark cycle was employed. The mice were given a conventional laboratory diet, as well as ample supplies of drinking water for feeding [9].

- *Administration of Doses for Acute Toxicity Test*

To study the acute toxicity of the methanolic extract from the leaves of *M. philippica*, 12 male albino mice were divided into four groups, each containing three animals.

Each group received a different dose: Group 1 was administered 50 mg/kg, Group 2 received 200 mg/kg, Group 3 was given 1000 mg/kg, and Group 4 received 2000 mg/kg.

Prior to dosing, the dorsal and flank areas of the mice were shaved to remove fur, exposing at least 10% of their total body surface. This preparation ensured good contact between the skin and the applied extract. The methanolic extract was then uniformly applied to the shaved areas of the skin.

To maintain contact and prevent the extract from being rubbed off, the treated areas were covered with a porous gauze dressing and secured with non-irritating tape. During the 24-hour exposure period, each mouse was housed

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul538

individually to prevent any oral ingestion of the test extract by other animals [9].

Following the 24-hour exposure, the mice were monitored for 14 days. During this observation period, the animals were closely observed for any signs of skin irritation or behavioral changes, allowing for a thorough assessment of the acute toxicity of the methanolic extract.

➢ *Ointment Formulation*

- *Ointment base (negative control) Preparation*

White wax was melted in a suitable dish on a warm water bath (65° to 70°C).

Table 1 Formulation of Ointment Base

Ingredients	Quantity
White wax	5g
White petrolatum	95g
Total	100g

- *M. Philippica Extract Ointment Formulation*

The ointment was prepared by mixing *M. philippica* extract and melted ointment base by stirring method to prepare a smooth paste, gradually incorporating more base

Table 2 *M. Philippica* Ointment Formulation

Ingredients	1%	5%	10%
M. philippica leaf extract	0.5g	2.5g	5g
Ointment base	49.5g	47.5g	45g
Total	50g	50g	50g

- *10% Povidone Iodine Ointment Formulation*

The ointment formulation was adopted from the study of Wuhan Diao Pharmaceutical Co. Ltd titled "Povidone iodine ointment and preparation method thereof".

The preparation method for the 10% povidone iodine ointment involves melting polyethylene glycol-400 and PEG-4000 at 78–82°C for 30 minutes with stirring, then adding xylitol and glycerol to the mixture and stirring for an additional 15 minutes while maintaining the temperature.

Table 3 Formulation of 10% Povidone Iodine Ointment

Ingredients	Quantity
Povidone iodine	5g
Polyethelyne glycol-400	12.5g
Potassium Iodate	0.15g
PEG-4000	12.5g
Xylitol	5g
Glycerol	8.5g
Sodium lauryl sulphate	0.5g
Purified water	5.85g
Total	50g

➢ *In Vivo Wound Healing Assay*

- *Excision Wound Model*

An excision wound is a type of wound created by surgically removing a portion of tissue from the skin, specifically targeting the epidermis (the outer layer) and part of the dermis (the layer below the epidermis). This model is commonly used in research to study the healing process and the effects of various treatments. Prior to preparing the wound site, each male albino mouse was anesthetized using EMLA Cream, a topical anesthetic that contains a combinationof lidocaine and prilocaine. EMLA Cream is preferred over systemic anesthesia or analgesics due to its localized effect, which provides targeted pain relief directly at the site of the procedure without affecting the animal's overall systemic function. This is especially important in small animals like mice, as it minimizes the risks associated with systemic anesthesia and is easy to apply. The strength of EMLA Cream that was used was 5%, with about 1 to 2 grams applied to the area being treated. For the excision wound, an area of approximately 314 mm² was delineated on the mice's skin and anesthetized using EMLA Cream, after 30 minutes of applying anesthesia, 6 mm of the skin was removed using Dermal Anchor Punch at the dorsal back of the mice. The mice were allowed to recover from the anesthetic effects before being returned to their cages, marking this as day zero (0) of the study. Treatment with various ointments began 24 hours post-wound creation, on day one, and continued daily until complete healing was achieved by day 14.

Add the white petrolatum and continue heating until liquefied. Removed from the heat and stirred the mixture until it begins to congeal [2]. Until homogeneous ointment is formed. This was then transferred in a suitable container [10].

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Separately, sodium lauryl sulphate was dissolved in purified water at 78–82°C while stirring for 15 minutes. The contents of both containers were then combined in a blender, stirred at 78–82°C for 15 minutes, cooled to 60–65°C, and mixed with potassium iodate solution and 10% povidone iodine powder using a homogenizer for 1–1.5 hours. Finally, the mixture was cooled, degassed, and formed into a ointment [17].

Table 4 The mice will be randomly divided into five groups, with five animals in each group

Group	Treatment
Group 1 (Positive Control)	Treated with 10% povidone iodine ointment
Group 2 (Negative Control)	Treated with nonmedicated ointment (base)
Group 3	Treated with low dose (1%) of *M. philippica* methanolic leaf extract ointment
Group 4	Treated with middle dose (5%) of *M. philippica* methanolic leaf extract ointment
Group 5	Treated with high dose (10%) of *M. philippica* methanolic leaf extract ointment

Positive control group is 10% povidone iodine ointment. This served as a reference standard for comparison. Group 2 received the negative control nonmedicated ointment (base). Group 3 received the ointment formulation containing low dose (1%) of *M. philippica* methanolic leaf extract. Group 4 received the ointment containing middle dose (5%) of *M. philippica* leaf extract. Group 5 received the ointment containing high dose (10%) of *M. philippica* leaf extract

The same formulation was applied topically on the wound site from the second day of wound creation up to its complete healing, was treated the animals once a day.

- *Wound Contractions*

The contraction of wound healing activity of *M. philippica* methanolic leaf extract shall be taken as the parameter for the wound contraction. The area of the wound created in each animal was traced on transparent sheet on day 2, day 4, day 6, day 8, day 10, day 12 and day 14 after creation of wound [4]. The area was measured with graph paper and calculated area percent of wound contraction was determined using the following formula:

$$\% \text{ Wound Contraction} = \frac{(\text{Initial Area} - \text{Final Area})}{\text{Initial Area}} \times 100$$

Table 5 Percentage Yield of the Methanolic Leaf Extract of *M. philippica*

Trial	Weight of dried leaves	Weight of crude extract	Percentage yield
1	100g	15.1g	15.1%
2	100g	15.6g	15.6%
3	100g	17.6g	17.6%
Mean Value	16.1%		

➢ *Confirmatory tests of the phytochemical constituents of methanolic leaf extract of M. philippica*

Ferric chloride test for tannins showed positive result, indicated by greenish black color. Alkaline reagent tests also showed positive result for flavonoids, producing a deep yellow coloration which disappeared on mixing with dilute acid, confirming the presence of flavonoids. The saponins test using foam test showed positive result as well for saponins, with foam formation indicating the presence of saponins.

➢ *Experimental Groupings*

The animals used to determine the potential wound healing activity of *M. philippica* (Doña Aurora) were albino male mice (8-10 weeks old and weighed 20-30 g).

In this formula, "Initial Area" refers to the area of the wound at the time of creation, while "Final Area" represents the area measured at the specified time points (on days 2, 4, 6, 8, 10, 12, and 14). This method allowed for a detailed assessment of the wound healing process over time.

➢ *Statistical Analysis*

Statistical analysis employed SPSS software version 27. The experimental results were obtained from excision wound models were expressed as mean and standard deviation. Results was compared with the corresponding control groups using one-way ANOVA. The data deem statistically significant at p value < 0.05. When data are statistically significant by one-way ANOVA, we used post hoc analysis using Tukey's Honest Significant Difference (HSD).

III. RESULTS AND DISCUSSIONS

➢ *Average percentage yield of M. philippica methanolic leaf extract*

The average percentage yield on triplicate analysis of 100g sample was 16.1%. Table 5 shows the individual yield obtained from each trial.

➢ *Ointment Formulations*

The ointment formulations of positive, negative and low, middle, and high dose of extract were shown in Fig. 1 10% Povidone iodine has reddish brown color and no odor. and negative control is plain white, while the low dose concentration of extract is light green in color, the middle dose concentration of extract is dark green and the high dose concentration of extract is greenish black, these three has no odor.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul538

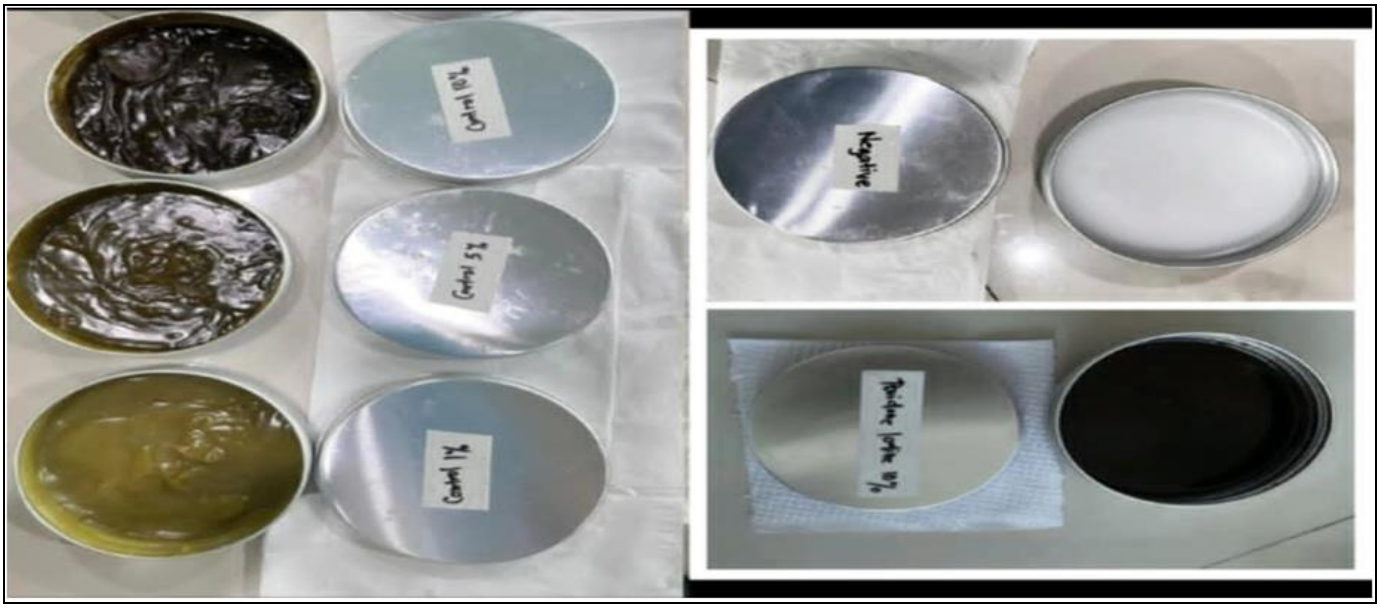

Fig 1 Ointment formulation of negative, positive (10% povidone iodine 10%), and 1% (low), 5%(middle), and 10% (high dose) of extract.

➢ *Acute Dermal Toxicity Test*

Acute Dermal Toxicity started at 200mg/kg. The four group (50mg/kg, 200mg/kg,1000mg/kg and 2000mg/kg), showed no noticeable behavioral changes, with no death after 24 hours. The four groups were further observed for 14 days.

Table 6 Result of Toxicity Testing with Group 1 (50mg/kg), Group 2 (200mg/kg), Group 3 (1000mg/kg), Group 4 (2000mg/kg) from March 12 to 25

Toxicity Testing	Alive	No Irritation	No Inflammation
Group 1	100%	0%	0%
Group 2	100%	0%	0%
Group 3	100%	0%	0%
Group 4	100%	0%	0%

➢ *Data Analysis*

Table 7 shows the average wound area measurements at different times, with notable healing trends between the various treatment groups. All groups started the study with the same wound size (28.27 mm^2) on Day 0, thereby establishing a common baseline for comparison purposes. The 10% extract group demonstrated the highest healing response, similarly with 5% with total wound closure by Day 14, and with minimal variability between subjects, as

Table 7 Descriptive Statistics Result for Wound Area

Time	10% Extract	5% Extract	1% Extract		
	Mean	SD	Mean	SD	Mean
Day 0	28.27	0	28.27	0	28.27
Day2	27.34	1.1	27.72	1.01	27.81
Day 4	26.97	0.83	27.62	0.42	25.86
Day 6	18.71	5.55	23.24	2.95	22.99
Day 8	8.39	5.71	10.97	3.51	10.18
Day 10	0.94	0.74	5.41	4.51	2.66
Day 12	0.03	0.01	0.27	0.36	0.48
Day 14	0.00	0	0	0	0.08

Table 8 shows the percentage contraction of the wound over the 14 days, thereby providing further insight into treatment effectiveness. The findings indicate that, although all active treatment groups eventually realized near-complete closure of the wound, their rates of contraction were significantly disparate. The 10% extract group recorded a rate of contraction equal to 96.67% on Day 10 and complete

There was no death after the end of the observation period, with no irritation and inflammation aside from that there were no behavioral changes like convulsion or tremors and physical change. Reflected by consistently low standard deviation values. The 5% and 1% extract groups demonstrated a more gradual but progressive healing response, with the 5% concentration being marginally superior to the 1% formulation. The positive control group demonstrated rapid early healing but then plateaued in the later stages, while the negative control group consistently demonstrated the poorest results, with incomplete healing still present at study termination. Closure on Day 14,

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul538

reflecting both velocity and consistency in its therapeutic action. The positive control realized identical final results but through a different pattern of healing, with a higher initial rate of contraction that reduced in subsequent phases. The 5% and 1% extract groups realized intermediate results, ultimately reaching similar endpoint results but after a longer time. Notably, the negative control group remained consistently behind the active treatments, thereby further validating the therapeutic effects of the experimental treatments.

Table 8 Descriptive Statistics Result for Percent of Contraction

TIME	10% EXTRACT		5% EXTRACT		1% EXTRACT		POSITIVE CONTROL		NEGATIVE CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DAY 0	0	0	0	0	0	0	0	0	0	0
DAY2	3.31	3.87	1.98	3.56	1.64	2.84	8.48	4.17	0	0
DAY 4	4.6	2.93	2.32	1.48	8.55	10.64	25.38	12.4	8.41	12.83
DAY 6	33.83	19.63	17.82	10.42	18.68	11.3	49.18	10.1	32.56	8.3
DAY 8	70.32	20.18	61.2	12.41	64.01	29.72	70.58	8.2	66.92	17.53
DAY 10	96.67	2.61	80.86	15.93	93.54	6.31	84.14	5.51	85.22	17.12
DAY 12	99.92	0.07	99.59	0.77	99.41	0.97	95.28	4.96	92.87	9.69
DAY 14	100	0	100	0	99.99	0.01	99.77	0.32	96.53	5.46

The Post Hoc Comparisons tests described in Table 9 provide important information for evaluating the effectiveness of treatments. Follow-up analyses revealed no statistically significant differences between the positive control and any of the extract concentrations, suggesting equal effectiveness between the standard treatment and the experimental samples. In contrast, compared to the negative control, both the 10% and 5% extracts demonstrated statistically enhanced performance with observed p-values of 0.023 and 0.039, respectively.

Table 9 Post Hoc Test Result of Percent of Contraction comparing between Control (Positive and Negative) and *M. philippica* concentration (1%, 5%, 10%) Extract Ointment

Post Hoc Comparisons - Group						
		Mean Difference	SE	df	t	p_{tukey}
Positive Control	Control 1% Ointment (Plant Extract)	3.025	21.715	35	0.139	1
	Control 10% Ointment (Plant Extract)	6.378	21.715	35	0.294	0.998
	Control 5% Ointment (Plant Extract)	8.698	21.715	35	0.401	0.994
Negative Control	Control 1% Ointment (Plant Extract)	3.265	21.715	35	0.15	0.660
	Control 5% Ointment (Plant Extract)	-2.408	21.715	35	-0.111	0.039
	Control 10% Ointment (Plant Extract)	-0.088	21.715	35	-0.004	0.023

- Note. P-value adjusted for comparing a family of 5 estimates.

Significance if p-value is less than 0.05 level of significance

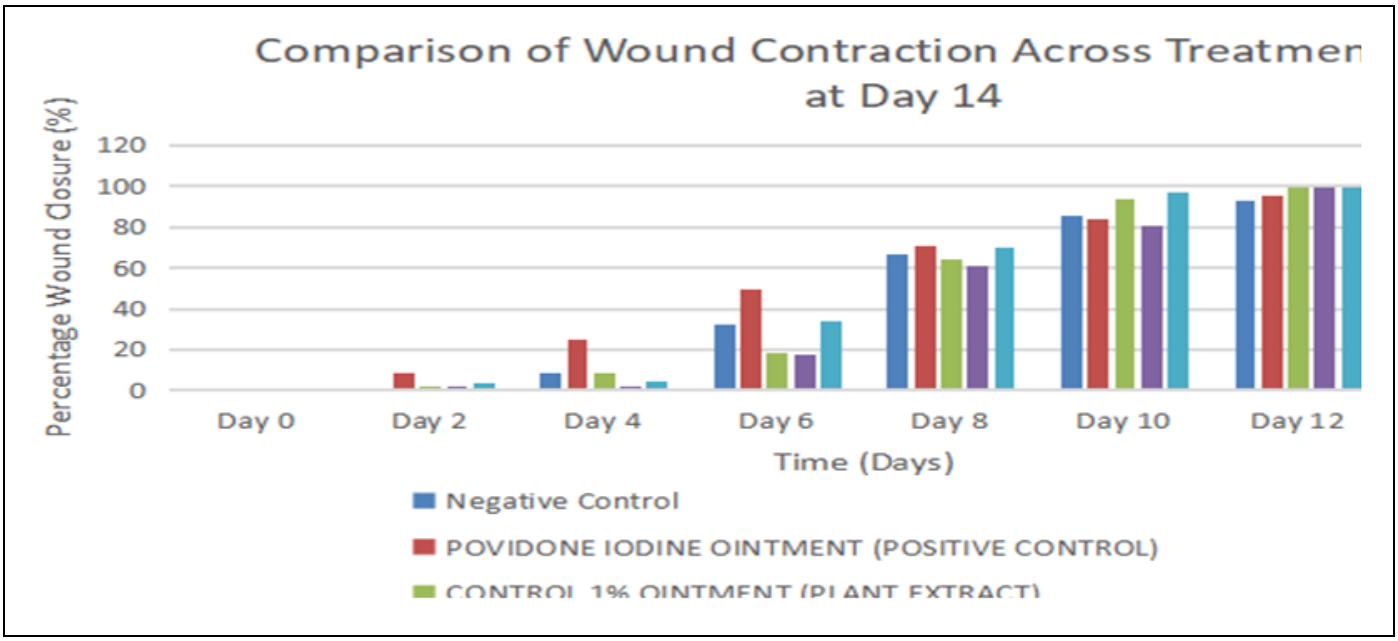

Fig 2 Comparison of Wound Contraction across Treatment Groups at Day 14

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul538

The 1% extract did not demonstrate improvement over the negative control, and the effect may be concentration-dependent. Overall, these findings suggest that the 10% extract formulation is the most promising therapeutic candidate, combining the effectiveness of standard treatments with the potential advantages of a natural product. The consistency of efficacy and enhanced healing in the 10% concentration justify further investigation into its mechanisms of action and potential clinical utility.

The bar chart illustrates comparison of wound closure across treatment groups. This graph allows one to see how the healing is going under different experimental conditions. Throughout Phase I (Days 0-2), the treatment groups are clustered together for wound closure, with the positive control having only a slight advantage (8.48% Day 2 closure). This 10% extract group begins improving steadily from Day 4 onwards with a continued increase in percentage closures. This group is surpassing both the 5% and 1% extract groups by Day 6 in healing, and the trend continues with increased measurements. The mid-stage (Days 8-10) shows evident differences among the treatment groups. The 10% extract achieves 96.67% closure on Day 10, superior to the positive control (84.14%) and the lower concentration treatments. This phase also shows response dependency on concentration, with the 5% treatment (80.86%) intermediate between the 10% and 1% (93.54%) treatments. The negative control is consistently behind all active treatments at this phase. Final measurements (Days 12-14) reveal the 10% extract to be the most potent of the three. It attains complete wound closure (100%) by Day 14. The positive control and the 5% extract get close to this level but attain slightly lower final closure percentages (99.77% and 100% respectively). The negative control is proof of imperfect healing (96.53%), providing a clear point of comparison.

IV. DISCUSSION

The study indicates that *M. philippica* methanolic leaf extract exhibit significant wound healing activity in the excision wound model using male albino mice. Among the different concentrations tested, the 10% extract ointment showed the most pronounced effect, promoting faster wound contraction and closure compared to 1% and 5% concentrations. The group treated with 10% extract ointment achieved a wound healing rate comparable to positive control group treated with 10% povidone iodine ointment with no statistically significant difference observed between them by the end of 14-day observation period. In contrast, the negative control group, which received only the ointment base, showed the slowest rate of wound. When comparing treatments, post hoc analysis indicated no statistically significant difference between 10% extract group and the positive control (10% povidone iodine), suggesting that the highest concentration of the plant extract is as effective as the standard treatment. However, both 10% and 5% extract groups performed significantly better than the negative control, with p-value 0.023 and 0.039, respectively, confirming that these concentrations of extract provide real therapeutic benefits beyond natural healing. The 1% extract did not show a statistically significant improvement over the negative control, indicating a dose-dependent effect.

The phytochemical analysis further supported these findings by confirming the presence of tannins, flavonoids, and saponins in the methanolic leaf extract-compound known for their anti-inflammatory, antimicrobial, and tissue regenerative properties. These result not only validate the traditional use of *M. philippica* leaves for wound management but also suggest that the methanolic leaf extract, especially at 10% concentration, could serve as a natural and effective alternative for wound care. The findings open new possibilities for the development of accessible, plant-based therapeutic agents for wound healing, although further research is needed to explore other parameters and potential application.

V. CONCLUSIONS

The study confirmed the *Muessaenda phlippica* methanolic leaf extract, particularly 5% and 10% concentrations, shows significant wound healing activity comparable to 10% povidone iodine. Its safety and phytochemical properties support its potential for alternative wound healing. The lack of dose-dependent differences suggest that the 1% concentration have potential wound healing, offering cost and safety advantages.

ACKNOWLEDGMENT

The researchers would like to begin by expressing their heartfelt gratitude to the Almighty God for His mercy, grace, and guidance throughout the completion of this research study. With His mercy, they were able to conduct the research efficiently. His favor and direction provided them with the necessary knowledge for this endeavor and brought forth several individuals who served as invaluable support systems, enhancing the depth of this study.

Special thanks go to Ms. Junnin Garay, RPh, CPh MS Pharm, the instructor of Research and Thesis Writing, for her insightful guidance, encouragement, and assistance in the development of this paper. Acknowledgment is also due to Mrs. Patricia B. Jarabe, RPh, the researcher's thesis adviser, for her unwavering support, guidance, and encouragement throughout this process. Furthermore, the researchers extend their appreciation to Sir Espencer Sala, RPh for his encouragement, guidance, and assistance in shaping this paper. The researchers are also grateful to their dear friends and classmates, who provided unwavering support during challenging times. Lastly, the researchers wish to express profound gratitude to their families, particularly their parents, for their moral, emotional, and financial support.

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ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul538

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