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Potential Wound Healing Activity of *Citrus micrantha Rut.* (Biasong) Ethanolic Peel Extract on Excised Cutaneous Wounds in Male Albino Mice

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Abstract: Many Citrus plants, such as C. reticulata, C. aurantium, C. aurantifolia, C. sinensis, C. maxima, C. medica, C. clementina, C. paradisi, and C. limon, are recognized for their wound healing properties [36] [8] [6] [2] [3]. However, there is a lack of research specifically focused on Citrus micrantha (Biasong)—a wild plant native to the Philippines, known for its culinary and medicinal applications [19]. Therefore, this study aims to evaluate the wound healing activity of the plant's peels using ethanol as the extraction solvent, focusing on excised cutaneous wounds in male Swiss albino mice. To accomplish this, toxicity testing based on OECD 402: Acute Dermal Toxicity guidelines was conducted before preparing the ethanolic peel extract at varying concentrations (1%, 5%, and 10%) and incorporating it into ointment formulations deemed safe for the test subjects. These formulations were topically applied to the dorsal area of the mice, with wound contraction measurements serving as the parameter to evaluate healing progress over a 14-day observation period. A significant difference in wound healing activity between treatment groups was most evident by Day 14, with the 10% extract showing the highest contraction (99.194%) and smallest final wound area (0.2278 mm^2), followed by the 5% extract (98.772%, 0.347 mm^2), 1% extract (98.678%, 0.37 mm^2), negative control (97.22%, 0.7854 mm^2), and positive control (94.616%, 1.522 mm^2). The study concluded that the application of C. micrantha ethanolic peel extract ointment promoted wound healing and demonstrated a significant improvement compared to the positive control (Povidone-Iodine ointment).

Keywords: *Ethanolic Peel Extract, Excised Cutaneous Wounds, Wound Healing Activity.*

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I. INTRODUCTION

Wounds, such as cuts, scrapes, punctures, and common abrasions in children, often result from physical trauma, chemical exposure, thermal damage, or microbial invasion, all of which pose significant health risks [9]. Although the skin's microbiome plays a protective role, open wounds compromise this barrier and increase susceptibility to infections, further complicating the healing process [26] [31] [34]. Healing typically begins when platelets initiate hemostasis by releasing clotting factors, forming a fibrin clot that triggers inflammation, tissue formation, and remodeling to restore skin integrity [12] [31].

In search of effective and safer wound treatments, many have turned to plant-based remedies, particularly Citrus species, which have been traditionally recognized for their therapeutic applications. According to the Global Biodiversity Information Facility (2024), Citrus plants belong to the Rutaceae family, and their peels are abundant in vitamins, dietary fibers, and bioactive compounds such as flavonoids, phenols, saponins, alkaloids, and tannins [10]. These constituents have demonstrated antioxidant, antimicrobial, and anti-inflammatory properties that help reduce inflammation, promote tissue regeneration, and prevent infections, thereby facilitating wound healing [1]. Several Citrus species have been proven effective in enhancing wound healing in various animal models: for

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instance, C. reticulata has been shown to heal burn wounds in mice [7] and incision wounds in rabbits [14]; C. aurantium and C. aurantiifolia have been effective in excision and gingival wound healing in rats and rabbits, respectively [6] [8]; and C. sinensis, C. maxima, C. medica, C. clementina, C. hystrix, C. paradisi, and C. limon have shown a range of wound healing and antimicrobial activities across in vitro and in vivo models [17] [15] [29] [2] [4] [25] [36] [30] [3]. These effects are attributed to their active compounds like tannins, flavonoids, phenols, saponins, terpenoids, and citronellal, which possess antibacterial, antioxidant, and anti-inflammatory properties beneficial to wound healing [18] [5] [20] [21].

Among these species is *Citrus micrantha* (commonly known as Biasong), a wild citrus plant native to the Philippines and traditionally used for culinary and medicinal purposes [19]. *Citrus micrantha* Wester, once considered a separate species found in the southern Philippines, is now recognized as a variety of *Citrus hystrix* DC., called *C. hystrix var. micrantha*. This change is based on genetic studies showing they are closely related, supporting their classification under the same species [23] [10]. However, despite its potential and phylogenetic relationship to other Citrus species, research on its bioactive constituents and therapeutic properties remains scarce. Thus, this study aimed to investigate the wound healing activity of ethanolic *C. micrantha* peel extract using an excision wound model in male Swiss albino mice, a strain selected for its genetic uniformity, lack of pigmentation for easier observation, and avoidance of hormonal variation seen in females [28]. By conducting a phytochemical screening and assessing its wound healing effects, the study sought to validate the traditional medicinal use of *C. micrantha*, fill the gap in existing research, and contribute to the growing body of knowledge on plant-based remedies for wound care [27].

II. MATERIALS AND METHODS

A. Animals

Twenty-five male albino mice (6-8 weeks old, weighing 20-25 g) were obtained from Aquatic Tiangge, a pet breeder shop in Zamboanga City, Philippines. The animals were housed in individual cages under controlled conditions of temperature (22°C (±3°C)), humidity at least 30%, and light (12 hr light/12 hr dark cycle) with ad libitum access to standard laboratory pellets and distilled water supply 1 week before and during the experiment.

B. Plant Materials

One sack of *Citrus micrantha* fruits, containing 430 pieces, were procured from a local market (G-241 Stall by Letecia M. Calubag), which had been sourced by them from their Barangay Abaga in Lala City, Lanao del Norte, Philippines. The fruits were washed with running water, peeled using a kitchen knife, air-dried in the shade at room temperature (25–30°C) for 33 days, pulverized using electric blenders "Kyowa Serial No. 134167" and "BOBI Electric Herb Grinder", sieved through 40 and 60 mesh sieves, and stored in a clean plastic container. The plant was identified and authenticated by a plant biologist at Biological Sciences Department in the College of Science and Mathematics, Mindanao State University— Iligan Institute of Technology, Tibanga, Iligan City.

C. Extract Preparation

Citrus micrantha peel extract was obtained by maceration method as described by Namutebi et al. (2024) [22]. The pulverized peels were extracted using 95% ethanol with a ratio of 1:5 at a room temperature in an orbital shaker at 50 rpm for 7 days. The extracts were filtered through Whatman paper 1 and evaporated the ethanol in a water bath at 40°C. The extracts were placed in a glass container and stored at 3°C to 5°C.

D. Phytochemical Screening

The phytochemical screening of the crude ethanolic peel extract was conducted by a chemist from the Chemistry Department of College of Science and Mathematics, Mindanao State University— Iligan Institute of Technology, Tibanga, Iligan City to identify the presence of various phytochemicals by employing standard protocols such as the following [16]:

- *Mayer's Test for Alkaloids:*

  Add a few drops of Mayer's reagent to 1 mL of ethanolic peel extract. Appearance of a yellowish-brown or white precipitate indicates the presence of alkaloids.

- *Shinoda Test for Flavonoids:*

  Add ten drops of dilute HCl and a piece of magnesium to 1 mL of ethanolic peel extract. Appearance of a deep pink color indicates the presence of flavonoids.

- *Tetra Acetic Acid Test for Phenols:*

  Treat 1 mL of lead tetra acetic acid solution with 0.5 mL of ethanolic peel extract. Appearance of a yellowish color indicates the presence of phenols.

- *Froth Test for Saponins:*

  Add a drop of sodium carbonate solution to 5 mL of ethanolic peel extract in a test tube, shake vigorously, and let rest for 5 minutes. Foam formation indicates the presence of saponins.

- *Ferric Chloride Test for Tannins:*

  Add 2 mL of 5% FeCl3 to 1 mL of the ethanolic peel extract samples. Appearance of a greenish-black or dark blue color indicates the presence of tannins

- *Horizon Test for Terpenoids:*

  Add 2 mL of trichloroacetic acid to 1 mL of the ethanolic peel extract. Appearance of a red precipitate indicates the presence of terpenoids.

- *Salkowski Test for Steroids:*

  Mix 1 mL of ethanolic peel extract with 1 mL of chloroform, then add a few drops of concentrated sulfuric acid. Appearance of a red color indicates the presence of steroids

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E. Acute Dermal Toxicity Test

Twelve male albino mice were divided into 4 groups, with 3 mice in each group, to evaluate the toxicity of the test chemical following the OECD guidelines for the testing of chemicals for 14 days. This test aimed to establish a safe dose range for further wound healing evaluations of the *C. micrantha* extract. The test chemical was prepared by incorporating the extract, at varying doses, into an ointment base composed of white wax and white petrolatum to facilitate absorption to the skin. Group 1 received *C. micrantha* preparation at 50 mg/kg, Group 2 at 200 mg/kg, Group 3 at 1000 mg/kg, and Group 4 at 2000 mg/kg. The procedure continued until a dose causing toxicity or a resulting in a maximum of 1 death was identified, or until no toxic effects observed at the highest dose or death occurred at the lowest dose [24].

F. Ointment Formulation

The formulation of 30 g ointments containing *C. micrantha* crude extract followed the procedure adapted from the UNC Eshelman School of Pharmacy (2024) [32]. As shown in Table 1, three concentrations of the extract, 1% (low), 5% (middle), and 10% (high), were prepared by integrating the crude extract into an ointment base composed of white wax and white petrolatum. The wax was melted over controlled heat, after which the petrolatum was added and fully liquefied. Upon achieving a uniform consistency, the specific concentration of the plant extract was mixed into the base. The ointments were then transferred to labeled jars and allowed to solidify at room temperature. The resulting ointments were characterized by smooth, semi-solid textures with a tea-like odor, varying in color from light brown (1%) to greenish-brown (10%) depending on extract concentration.

Table 1 Ingredients and Proportions of the High-, Middle-, and Low-Concentration Extract Ointments

Ingredients	**Low Concentration (1%) Extract Ointment**	**Middle Concentration (5%) Extract Ointment**	**High Concentration (10%) Extract Ointment**
Crude Extract	0.3g	1.5g	3g
White Wax	1.485g	1.425g	1.35g
White Petrolatum	28.215g	27.075g	25.65g
Total	30g	30g	30g

The formulation of a 30 g ointment containing the 10% Povidone-Iodine, as shown in Table 2, followed the procedure adapted from Wuhan Diao Pharmaceutical Co. Ltd. (2023) [33]. Polyethylene glycol-400 and polyethylene glycol-4000 were heated together at 78–82°C for 30 minutes with continuous stirring. Separately, xylitol and glycerol were mixed and stirred for 15 minutes before being added to the first mixture, maintaining the same temperature for another 15 minutes. Meanwhile, sodium lauryl sulfate and purified water were combined and stirred at 78–82°C until dissolved. All mixtures were then combined and stirred until homogeneous, cooled to 60–65°C, and gradually mixed with potassium iodate solution and povidone-iodine. The final product was stirred until fully blended, cooled, and transferred into labeled ointment jars

Table 2 Ingredients and Proportions of the 10% Povidone Iodine Ointment

Ingredients	**Quantity**
Polyethylene glycol-400	7.5g
Polyethylene glycol-4000	7.5g
Xylitol	3g
Glycerol	5.1g
Sodium lauryl sulfate	0.3g
Purified water	3.51g
Potassium iodate	0.09g
Povidone iodine	3g
Total	30g

The formulation of a 30 g white ointment, as shown in Table 3, followed the procedure adapted from the UNC Eshelman School of Pharmacy (2024) [32]. White wax was melted on a hot plate, keeping the temperature between 70–75°C. Once fully melted, petrolatum was added and the mixture was heated until completely liquefied. Afterward, the mixture was removed from the heat and stirred during congealing to ensure a uniform consistency. The finished ointment was transferred to tightly sealed, labeled jars for proper storage and traceability throughout the experiment.

Table 3 Ingredients and Proportions of White Ointment

Ingredients	**Quantity**
White wax	1.5g
White petrolatum	28.5g
Total	30g

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G. In Vivo Wound Healing

➢ *Excision Wound Model*

Twenty-five male albino mice were divided into 5 groups, with 5 mice in each group, to evaluate the wound healing of *C. micrantha*. Before wound induction, the dorsal area of each mouse was shaved to expose the skin, and a 6-mm full-thickness circular excised wound was created by using biopsy punch and under local anesthesia using 5% EMLA cream [6]. Group 1 received 10% Povidone-Iodine (positive control), Group 2 received white ointment (negative control), and Groups 3A, 3B, and 3C received *C. micrantha* ointment at 1%, 5%, and 10%, respectively.

➢ *Administration of Sample Materials*

Topical application of the ointments was carried out once daily for 14 days. Prior to each application, wounds were gently cleansed using sterile cotton wool. The ointments were applied in a uniform thin layer directly over the wound site. Each mouse was housed individually to prevent interference from other animals and to minimize oral ingestion or cross contamination. At the end of each exposure day, excess ointment was removed, and the wounds of the test animals were measured using a Vernier scale and observed for wound contraction.

➢ *Wound Contraction Measurement*

Each wound was measured daily from Day 0 to Day 14 using a Vernier scale, with the wound areas recorded in square millimeters (mm^2), and the percent wound contraction calculated using the following formula [35]:

$$\%\ Wound\ Contraction = \frac{WA_0 - WA_t}{WA_0} x100$$

Where, WA_0 = wound area on Day 0, and WA_t = wound area on a given day (t).

This formula quantifies the reduction in wound size over time, reflecting the rate of healing and tissue regeneration among different treatment groups.

H. Data Collection and Statistical Treatment

Data collection focused on daily wound measurements and observational records of wound appearance and healing progression. Descriptive statistics, including mean, standard deviation, and standard error, were calculated using Microsoft Excel and R programming software. To assess the statistical significance of differences in wound healing among treatment groups, the Kruskal-Wallis test a non-parametric alternative to ANOVA was employed. A p-value of less than 0.1 was considered statistically significant. This statistical approach was chosen to accommodate non-normal data distributions and small sample sizes, enabling valid comparisons between the extract-treated, positive control, and negative control groups.

III. RESULTS AND DISCUSSIONS

A. Average Percentage Yield of C. Micrantha Ethanolic Peel Extract

Table 4 shows the percentage yield of the crude ethanolic peel extract of *C. micrantha* from three 20g samples. The yields were 3%, 3.4%, and 3.6%, with an average yield of 3.33%, indicating slight variations in extraction efficiency.

Table 4 Percentage Yield of C. Micrantha Ethanolic Peel Extract

Samples	Weight of the Powdered Peels	Weight of the Crude Extract	Percentage Yield
1	20g	0.6g	3%
2	20g	0.68g	3.4%
3	20g	0.72g	3.6%
Mean Value			3.33%

B. Phytochemical Screening of C. Micrantha

Phytochemical screening of the ethanolic peel extract of C. micrantha confirmed the presence of various secondary metabolites. As shown in Table 5, positive results were observed for tannins (greenish-black color), flavonoids (deep pink color), phenols (yellowish color), saponins (foam formation), and alkaloids (yellowish-brown), all in large amounts, while terpenoids (red precipitate) and steroids (red color) were present in moderate amounts. These findings support the hypothesis that *C. micrantha* peel extract possesses compounds that contribute to its observed wound healing activity and justify its formulation into a topical therapeutic ointment.

Table 5 Phytochemical Screening of the 95% Ethanol Peel Extract of *C. Micrantha*

Constituents	Results
Alkaloids	+++
Flavonoids	+++
Phenols	+++
Saponins	+++
Tannins	+++
Steroids	++
Terpenoids	++

Note: (-) = absence; (+) = trace; (++) = moderate; (+++) = large amounts of phytochemicals

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C. Acute Dermal Toxicity Test

The acute dermal toxicity test of *C. micrantha* ethanolic peel extract was conducted using OECD Guideline 402 with four dosage groups: Group 1 (50 mg/kg), Group 2 (200 mg/kg), Group 3 (1000 mg/kg), and Group 4 (2000 mg/kg). Results in Table 6 revealed that all test animals survived without any signs of skin irritation or inflammation, indicating 100% survival and no adverse reactions across all groups after 24 hours and even after further observation of 14 days. These results suggest that the extract is non-toxic and safe at the tested concentrations, supporting its potential for further use in wound healing studies.

Table 6 Results of Acute Dermal Toxicity Test

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%

D. Ointment Formulations

In Fig. 1, the extract concentrations of high (10%), middle (5%), and low (1%) used in the treatment ointments were based on the study by Goldenheim (1993) [11]. The high concentration extract ointment had a darker greenish-brown color, the middle concentration extract ointment had a dark greenish-brown color, and the low concentration extract ointment was light brown. Despite the color variations, all ointments had a tea-like odor, a homogenous texture, and a smooth semi-solid consistency.

Fig 1 High (10%), Middle (5%), and Low (1%) Extract Concentration Ointments Formulated with C. Micrantha

E. Data Analysis

Table 7 presents the wound area reduction trends in male albino mice treated with different concentrations of *C. micrantha* ethanolic peel extract and control ointments over 14 days. For the 1% extract group, no healing occurred during Days 1 to 7 as the wound area remained at 28.274 mm², but starting Day 8, a gradual decrease was noted down to 24.81 mm², then to 8.17 mm² by Day 10, 1.98 mm² by Day 12, and 0.37 mm² by Day 14, as well as with low standard deviations indicating consistent healing among mice. The 5% group similarly showed no reduction in the first week, but the wound area began decreasing on Day 8 (26.5462 mm²), then dropped significantly to 21.6768 mm² on Day 9 and 6.9117 mm² on Day 11, reaching 0.347 mm² by Day 14, and moderate variability was observed during Days 9–11, as reflected in increased standard deviations, which later stabilized. The 10% extract group also showed no early healing until Day 7, when the area started shrinking from 28.274 mm² to 26.546 mm², followed by a gradual decrease to 24.033 mm² by Day 9, then a sharp reduction to 8.796 mm² on Day 10 and 0.2278 mm² by Day 14. Standard deviations peaked during Days 8 to 10, indicating variability in early response, but decreased thereafter, suggesting more uniform healing. These findings confirm that higher concentrations of *C. micrantha* extract induced faster and more effective wound contraction, with consistent results across subjects in later stages of treatment. Meanwhile, the positive control group treated with 10% Povidone-Iodine ointment began showing wound contraction by Day 6, with the area steadily decreasing to 1.522 mm² by Day 14. The standard deviation increased from Day 6 onward, reflecting some variability in individual healing responses. In contrast, the negative control group treated with white ointment exhibited slower and less consistent healing, with wound contraction starting on Day 5 and reaching 0.7854 mm² by Day 14. While the standard deviations were initially higher, they gradually stabilized toward the end of the observation period. These results highlight that although natural healing occurred, it was less efficient and more variable compared to the extract-treated and positive control groups.

Table 7 Descriptive Statistics Result for Wound Area

Day	1% Extract		5% Extract		10% Extract		Positive Control		Negative Control	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
0	28.274	0	28.274	0	28.274	0	28.274	0	28.274	0
1	28.274	0	28.274	0	28.274	0	28.274	0	28.274	0
2	28.274	0	28.274	0	28.274	0	28.274	0	28.274	0
3	28.274	0	28.274	0	28.274	0	28.274	0	28.274	0
4	28.274	0	28.274	0	28.274	0	28.274	0	28.274	0
5	28.274	0	28.274	0	28.274	0	28.274	0	24.8184	4.73
6	28.274	0	28.274	0	28.274	0	26.5462	3.86347	18.2212	3.16

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7	28.274	0	28.274	0	26.546	3.863	26.5462	3.86347	15.7848	3.6
8	24.8184	4.7317	26.5462	3.8634	25.1324	7.024	25.1324	7.02483	12.1722	5.3257
9	16.8	3.87	21.6768	6.678	24.033	9.483	25.1324	7.02483	11.781	5.179
10	8.1684	2.45	16.336	7.37	8.796	6.2929	19.949	5.57	6.8884	1.758
11	4.71256	2.1511	6.91168	4.17	4	2.04	12.8804	4.463	4.71256	2.15
12	1.98	1.089	2.94676	1.89	2.199	1.29	7.7364	2.857	3.1416	0
13	0.545	0.219	0.95	0.81359	0.636	0.51279	3.988	2.28	1.72788	1.29
14	0.37386	0.2989	0.347	0.4789	0.22778	0.3154	1.522	1.938	0.7854	0

Table 8 presents the percent wound contraction trends in male albino mice treated with different concentrations of *C. micrantha* ethanolic peel extract and control ointments over 14 days. From Day 1 to Day 7, no wound contraction was observed across all tested concentrations of *C. micrantha* ethanolic peel extract, with a mean percent contraction of 0% and standard deviation of 0, indicating no measurable healing activity during the first week. Starting Day 8, all concentrations showed progressive improvement: 1% extract exhibited a mean contraction of 12.22%, 5% showed 6.11%, and 10% recorded 11.112%, with contraction rates continuing to increase until Day 14, nearing complete wound closure. Notably, the 10% concentration achieved the highest contraction by Day 14 (99.194%), followed by 5% (98.772%) and 1% (98.678%), demonstrating dose-responsive effectiveness. Standard deviations were higher during the early healing stages (Days 8–11), especially at 10%, reflecting variability in individual responses, but declined as wounds neared full closure, indicating more consistent outcomes in the final days. These results confirm that *C. micrantha* extract, particularly at higher concentrations, effectively promotes wound healing with increasing uniformity over time. Meanwhile, the positive control group treated with 10% Povidone-Iodine ointment showed no wound contraction from Day 1 to Day 5 (0% mean), indicating a delayed start to visible healing. Slight progress began on Days 6 and 7 with 6.11% contraction, but the high standard deviation (13.66) suggested considerable individual variation. Healing accelerated from Day 10 onward, with contraction increasing to 29.442%, then 54.446% by Day 11, and continued steadily to 94.616% by Day 14. The decreasing standard deviations over time, such as 6.85 on Day 14, indicate more consistent healing as the wounds neared closure. In contrast, the negative control group treated with plain white ointment exhibited the earliest signs of healing on Day 5 with 12.22% contraction and a high standard deviation (16.739), reflecting uneven responses among subjects. Wound closure progressed more slowly, reaching only 56.95% by Day 8 and 97.22% by Day 14, with fluctuations in standard deviations throughout, though these stabilized toward the end. These findings suggest that while natural healing occurred in the absence of active agents, it was slower and more inconsistent than in the treated groups, reinforcing the effectiveness of the extract and positive control.

Table 8 Descriptive Statistics Result for Percent of Contraction

Day	**1% Extract**		**5% Extract**		**10% Extract**		**Positive Control**		**Negative Control**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	12.22	16.739
6	0	0	0	0	0	0	6.11	13.66	35.552	11.18
7	0	0	0	0	6.11	13.66	6.11	13.66	44.172	12.75
8	12.22	16.7	6.11	13.66	11.112	24.84	11.112	24.84	56.95	18.838
9	40.554	13.69	23.332	23.6199	15	33.54	11.112	24.84	58.334	18.322
10	71.112	8.69	42.218	26.09	68.888	22.259	29.442	19.7	75.638	6.217
11	83.334	7.6	75.556	14.74	85.834	7.227	54.446	15.789	83.334	7.607
12	92.974	3.85	89.578	6.7	92.222	4.5625	72.64	10.1	88.89	0
13	98.072	7.77	96.64	2.8766	97.75	1.812	85.894	8.075	93.888	4.5625
14	98.678	1.058	98.772	1.6939	99.194	1.116	94.616	6.85	97.22	0

Table 9 presents the Kruskal-Wallis test results comparing the percent wound contraction among groups treated with varying concentrations of *C. micrantha* ethanolic peel extract (1%, 5%, and 10%), a positive control (10% Povidone-Iodine), and a negative control (white ointment). Several pairwise comparisons yielded p-values below 0.1, indicating marginal statistical significance: low dose vs. middle dose (p=0.07211), low dose vs. positive control (p=0.07291), middle dose vs. positive control (p=0.07291), and high dose vs. positive control (p=0.08275). These findings suggest that some extract-treated groups, particularly the middle concentration,

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exhibited wound contraction outcomes comparable to the standard treatment. Meanwhile, comparisons involving the negative control and among extract concentrations showed p-values above 0.1, reflecting no significant differences. Overall, the statistical results highlight the therapeutic potential of *C. micrantha*, especially at 5%, in promoting wound healing.

Table 9 Kruskal-Wallis Test Result of Percent of Contraction Comparing between Control (Positive and Negative) and *C.* Micrantha Concentration (1%, 5%, and 10%) Extract Ointment

Kruskal-Wallis Test result	**p-value**
Low Dose vs. Middle Dose	0.07211*
Low Dose vs. High Dose	0.1118
Low Dose vs. Positive Control	0.07291*
Low Dose vs. Negative Control	0.2237
Middle Dose vs. High Dose	0.1118
Middle Dose vs. Positive Control	0.07291*
Middle Dose vs. Negative Control	0.2237
High Dose vs. Positive Control	0.08275*
High Dose vs. Negative Control	0.2237
Positive Control vs. Negative Control	0.2237

Note: Significant if p-value < 0.1* p-value < 0.05** and p-value < 0.01***

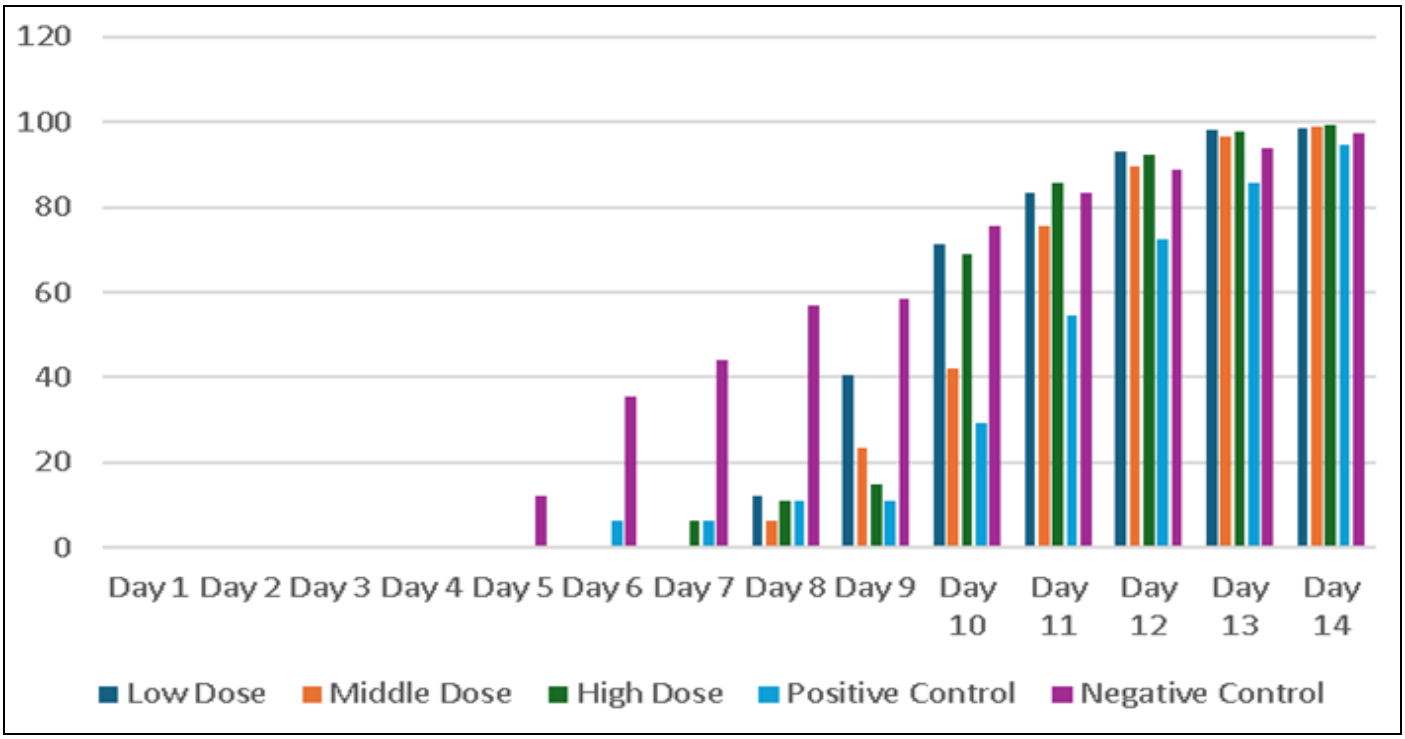

Fig 2 Bar Graph Result of Percent of Contraction of C. Micrantha Ethanolic Peel Extract Concentration (1%, 5%, 10%) and Control Groups (Positive and Negative)

Fig. 2 presents the wound contraction outcomes of male albino mice treated with varying concentrations of *C. micrantha* ethanolic peel extract (1%, 5%, 10%), alongside positive (10% povidone-iodine) and negative (ointment base) control groups over 14 days. The 10% extract group achieved the most rapid and consistent healing, closely followed by the 5% group, both showing significant contraction starting the second week. The 1% group showed delayed healing but reached near-complete contraction by Day 14. The positive control demonstrated moderate improvement, while the negative control showed the slowest and most variable healing. These findings indicate a dose-dependent wound healing effect of *C. micrantha*, with higher concentrations promoting faster and more consistent wound healing.

IV. DISCUSSION

The study began with the extraction of C. *micrantha* ethanolic peel extract, which yielded an average of 3.33% from three 20g samples, showing minor variation in extraction efficiency. Phytochemical screening confirmed the presence of secondary metabolites such as tannins, flavonoids, phenols, saponins, alkaloids (in large amounts), and moderate levels of terpenoids and steroids, all known for their roles in wound healing [1]. Acute dermal toxicity testing, based on OECD Guideline 402, showed 100% survival and no skin irritation in all dose groups (50–2000 mg/kg), indicating the extract's safety for topical application. Ointments were formulated using 1%, 5%, and 10% extract

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concentrations, with all preparations exhibiting consistent texture, homogeneity, and a tea-like odor, despite color differences. Wound healing analysis revealed that all extract-treated groups had no contraction during the first week, but healing accelerated significantly afterward, particularly in the 10% group, which achieved the highest contraction by Day 14, followed by the 5% and 1% groups. Positive controls (10% povidone-iodine) showed delayed but steady healing, while the negative control group (ointment base) showed the slowest and most inconsistent results. Percent contraction data supported these trends, with all extract groups nearing full wound closure by Day 14. Statistical analysis using the Kruskal-Wallis test suggested marginal significance ($p < 0.1$) in some group comparisons, notably between the extract-treated groups and the positive control, highlighting the potential of *C. micrantha*, especially at 5% and 10% concentrations, as a promising natural wound healing agent.

V. CONCLUSION

This study successfully achieved its objectives in evaluating the wound healing potential of *Citrus micrantha* ethanolic peel extract. The extract yielded an average of 3.33%, indicating moderate but consistent extraction efficiency. Phytochemical screening confirmed the presence of important bioactive compounds such as tannins, flavonoids, phenols, saponins, terpenoids, alkaloids, and steroids, which are known to promote wound healing. Toxicity testing showed no signs of irritation or mortality up to 2000 mg/kg, confirming the extract's safety for topical application. The formulated ointments containing 1%, 5%, and 10% extract concentrations demonstrated effective wound healing activity, with the 10% concentration producing the most significant and consistent results, comparable to the positive control (povidone-iodine). Statistical analysis confirmed significant differences between the extract-treated groups and the control groups, highlighting a dose-dependent effect. Overall, the findings suggest that *C. micrantha* ethanolic peel extract is a safe, effective, and promising natural alternative for topical wound care.

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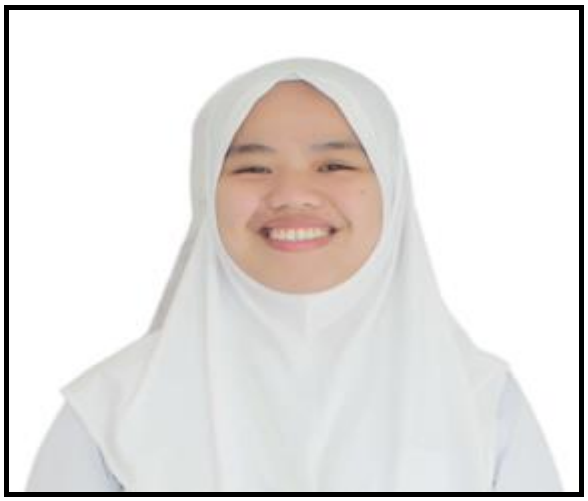

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