

## Wound-healing potential of topical application of preparations from Noni (*Morinda citrifolia* L.) leaf extract

Ly Hai Trieu<sup>1</sup>, Dao Nguyen Nhu Quynh<sup>1</sup>, Nguyen Thi Kim Oanh<sup>2</sup>, Lam Bich Thao<sup>1</sup>,  
Nguyen Minh Khoi<sup>3\*</sup>, Tran Ba Hieu<sup>4</sup> and Le Van Minh<sup>1\*</sup>

<sup>1</sup>Research Center of Ginseng and Medicinal Materials (CGMM), National Institute of Medicinal Materials,  
41 Dinh Tien Hoang Street, Ben Nghe Ward, District 1, Ho Chi Minh City 70000, Vietnam

<sup>2</sup>Saigon Pharmaceutical Sciences and Technologies Center, University of Medicine and Pharmacy at Ho Chi Minh City,  
41 Dinh Tien Hoang Street, Ben Nghe Ward, District 1, Ho Chi Minh City 70000, Vietnam

<sup>3</sup>National Institute of Medicinal Materials, 3B Quang Trung Street, Hoan Kiem District, Ha Noi, 10000, Vietnam

<sup>4</sup>Institute of Biomedicine and Pharmacy, Vietnam Military Medical University, 222 Phung Hung Street,  
Ha Dong District, Hanoi 10000, Vietnam

Received 29 July 2021; revised received 22 May 2023; accepted 29 May 2023

Developing products derived from medicinal herbs with wound-healing effects has been a current trend. This study was set up to observe the wound healing efficiency of herbal preparations formulated from *M. citrifolia* leaf extract on mice. The wound healing activity of cream, ointment, and powder preparations containing Noni leaf extract of 1% and 5% (w/w) was investigated on the excision wound model. The potential preparation was continuously evaluated for rabbit skin irritation, antioxidant activity, total flavonoid, and rutin contents. Results revealed that the topical application of Noni leaf extract preparations treated groups increased remarkably wound contraction during 11 days of treatment. A cream containing 1% extract not only had the ability to significantly reduce the size of the wound but also regenerated the structure of the skin histology at the wound area. Moreover, the cream showed safety following an acute dermal irritation study on rabbits as well as an antioxidant effect via inhibition of DPPH and ABTS free radicals. The present study also identified the content of total flavonoids and rutin markers in the cream. This research affirms the topical application of the cream containing *M. citrifolia* leaf extract as a known wound healing factor from traditional medicine.

**Keywords:** Cream, *Morinda citrifolia* leaves, Ointment, Powder, Wound healing activity

**IPC code; Int. cl. (2021.01)-** A61K 36/00, A61K 36/74, A61K 127/00, A61P 17/00

### Introduction

The human skin is the largest organ of the body and plays an important role in protecting the body against adverse external influences. The skin is constantly exposed to potential injuries, like acute injuries or chronic wounds, which interfere with and disrupt the skin's integrity, change its physiologic condition and result in wound formation. In this case, a cascade of biological processes begins to regenerate normal-tissue anatomy and ensure wound contraction<sup>1</sup>. The normal wound healing process involves three overlapping but distinct phases of hemostasis/inflammation, proliferation, and remodeling. The duration time of this process, as well as the scarring probability, depends on the severity of the wound and each person's healing

ability<sup>2</sup>. The first stage involves the activation of platelet aggregation to form blood clots, which takes place due to the intervention of neutrophils and macrophages. The following phase proliferative is characterized by the restoration of the vascular network and formation of granulation tissue, followed by fibroblast proliferation, connective tissue and capillary formation, epithelialization, and finally result in wound contraction. Lastly, the remodelling phase helps to restore tissue integrity and function, accelerate wound regeneration, and determines the shape of the wound after the process is complete<sup>2-4</sup>.

Even though wound healing is a natural bodily response and capable of self-healing, however, restore integrity and avoid adverse external influences requires rapid wound healing. There are many strategies studied and applied in the treatment of wound healing. In recent years, there is an increasing

\*Correspondent author

Email: lvminh05@gmail.com; lvminh\_hcm@nimm.org.vn

demand for the use of herbal extracts in treatments. These agents are gaining popularity worldwide as a rich and inexpensive variety of natural origin ingredients with fewer side effects, more health benefits and hypersensitivity reactions rarely occur<sup>5</sup>. Since a variety of botanical medicines have been traditionally used to enhance wound repair as references, studies have demonstrated that many plant extracts and their active components can accelerate the wound closure process<sup>6</sup>.

*Morinda citrifolia* (family Rubiaceae), commonly known as Noni is one of the folk herbs that has been used decades ago to treat many diseases. Noni has been reported to have a broad range of therapeutic and nutritional value<sup>7</sup>. Nearly all the parts obtained from the plant have been traditionally used for the therapeutic treatment of numerous diseases<sup>8</sup>. Specifically, Noni fruit helps ease digestion, is a laxative, and treatment of hemorrhages, colds, asthma, tendon pain, and diabetes. For the leaves, crushed Noni leaves can treat acne, quicken skin regeneration, cure dysentery, malaria, and tonic through the oral route. Noni roots can be used to make alcohol, as well as medicine to treat high blood pressure, rheumatoid arthritis, and back pains. Moreover, Noni is also used as a folk remedy that treats broken bones, deep cuts, bruises, sores, and wounds<sup>7</sup>.

Many studies of *M. citrifolia* have revealed the existence of more than 200 phytochemical substances from different parts of the plant such as anthraquinones, triterpenoids, flavonoids, iridoids, lignans, sterols, and vitamins with identified and isolated bioactive properties<sup>8,9</sup>. Many pharmacological effects of Noni have been reported as immunostimulatory effects, antioxidant, antitumor, antiseptic, antimicrobial, analgesic, neuroprotective, antiallergic, antiangiogenic, anti-gastric ulcer and oesophagitis<sup>10-12</sup>. In addition, Noni juice has been designated for treating chronic pathological conditions, namely cancer, diabetes mellitus, cardiovascular diseases, and inflammatory disorders. Furthermore, it also possesses wound healing activity, gout and hyperuricemia healing<sup>11</sup>.

Since *M. citrifolia* leaves have been traditionally used for wound healing activity, much research had been carried out to illustrate their healing effectiveness. Studies proved that the Noni leaf extract possesses antioxidant activity and enhances wound healing, such as an increase in wound

contraction, tensile strength, granulation tissue weight, collagen and hydroxyproline content, as well as a decrease in epithelialization time<sup>13,14</sup>. In addition, some chemical compounds present in Noni leaves might be involved in its wound healing process, for example, saponins and terpenoids with antibacterial activity, tannins with stopping bleeding capacity, iridoid glycosides as anti-inflammatory, and flavonoids have significant antimicrobial, antioxidant and anti-inflammatory effects<sup>8,15</sup>. Prior studies demonstrated that rutin is one of the main flavonoids found in *M. citrifolia* leaf<sup>8</sup>. Rutin compound exhibits anti-inflammatory, antimicrobial, antioxidant, anticoagulant, immunomodulatory and wound healing<sup>10,16</sup>. Rutin was proven to promote wound healing and reduce the risk of wound ulcers by preventing oxidative stress and inflammatory responses<sup>17</sup>. Furthermore, our previous study demonstrated that 70% ethanol extract from *M. citrifolia* leaves effectively promoted wound healing in mice. Following on from the previous study, the objective of the present study is to formulate preparations of *M. citrifolia* leaf extract, evaluate their wound healing efficacy, safety test by acute dermal irritation/corrosion study, determination of total flavonoid and rutin contents, in order to develop the appropriate formulation for product development that supports the treatment of human wounds.

## Materials and Methods

### Formulation of herbal preparations

Standardized extract from *M. citrifolia* leaves as well as its preparation forms including creams, ointments, and powders were provided by the Chemicals-Natural Preparations Department Research Center of Ginseng and Medicinal Materials Ho Chi Minh City. *M. citrifolia* leaf extract was extracted according to an optimized extraction process<sup>3,18</sup>. Our previous study showed that *M. citrifolia* leaf extract at concentrations of 1 and 5% had wound healing effect<sup>3</sup>. Therefore, the medicated preparations were prepared by incorporating different quantities of the standardized extract into the prepared base to get 1 and 5% (w/w) of the standardized extract in preparations. The formulation of herbal preparations is given in Table 1.

### Chemicals and reagents

Several chemicals and reagents were used in this study such as gentamicin sulfate ointment 0.1%

Table 1 — Ingredients of herbal formulations

Extract concentration	Cream	Ointment	Powder
0% (Base)	Vaseline: 18 g White soft paraffin: 42 g Hard Paraffin: 26 g Cetostearyl alcohol: 14 g Total: 100 g	Tween 80:25 g Vaseline: 75 g Total: 100 g	Talcum powder: 100 g Total: 100 g
1%	Tween 80: 20 g Extract: 1.2 g Base: 78.8 g Total: 100 g	Tween 80:25 g Extract: 1.2 g Vaseline: 73.8 g Total: 100 g	Talcum powder: 98.8 g Extract: 1.2 g Total: 100 g
5%	Tween 80: 20 g Extract: 6 g Base: 74 g Total: 100 g	Tween 80:25 g Extract: 6 g Vaseline: 69 g Total: 100 g	Talcum powder: 94 g Extract: 6 g Total: 100 g

Note: Including extract moisture (20%)

(Japan), acetonitrile, formic acid, methanol (Merck), formaldehyde (Himedia), rutin standard (HPLC  $\geq$  98%), DPPH reagent (1,1-diphenyl-2-picrylhydrazyl), ABTS reagent (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and ascorbic acid were purchased from Sigma-Aldrich® Co. Ltd (USA), and some other analytical chemicals.

#### Animals

Healthy *Swiss albino* male white mice, 5-6 weeks old with an average weight of  $20 \pm 2$  g, and healthy New Zealand adult female rabbits (not pregnant or breastfeeding), 10-12 weeks old, weighing 2.0-2.5 kg, were obtained by The Institute of Vaccines and Medical Biologicals in Nha Trang City. The mice were grown in  $33 \times 21 \times 15$  cm plastic cages, supplied with food in pellets and adequate drinking water, and raised and allowed to stabilize for at least one week before the experiment. The rabbits were kept separately in each cage and stabilised for 5 days before the experiment. The experiments were carried out in inappropriate room conditions at 25–30°C, relative humidity at 30-70%, and guaranteed an adequate amount of light for 12 hours daily. Studies using these animals strictly adhere to the principles stated in the Guide for Care and Use of Laboratory Animals.

#### Investigation of wound healing efficacy of preparations

##### Wound excision model on mice

A wound excision model on mice was performed according to the previously described protocol with slight modifications<sup>3</sup>. The model was carried out in order to evaluate the wound healing efficacy of the preparations from Noni leaf extract by observing and measuring the percentage of wound contraction. Mice

were anaesthetized before and during the wound-cutting procedure. A determined area on the back of the mouse was shaved, creating a wound by using sharp, sterilized laboratory tools with a diameter of 7-9 mm and 0.5-1.0 mm depth. After the wound excision, the mice were kept separately in  $28 \times 17 \times 13$  cm plastic cages to prevent infection. Mice were randomly divided into eleven groups of 6 ( $n = 6$ ) as follows:

+ Group 1: Mice were applied with a cream foundation.

+ Group 2: Mice were topically treated with cream containing 1% (*w/w*) Noni leaf extract.

+ Group 3: Mice were topically treated with cream containing 5% (*w/w*) Noni leaf extract.

+ Group 4: Mice were applied with an ointment foundation.

+ Group 5: Mice were topically treated with an ointment containing 1% (*w/w*) Noni leaf extract.

+ Group 6: Mice were topically treated with an ointment containing 5% (*w/w*) Noni leaf extract.

+ Group 7: Mice were applied with powder foundation.

+ Group 8: Mice were topically treated with powder containing 1% (*w/w*) Noni leaf extract.

+ Group 9: Mice were topically treated with powder containing 5% (*w/w*) Noni leaf extract.

+ Group 10: Positive control group, mice were topically treated with 0.1% Gentamicin.

+ Group 11: Negative control group, mice were applied with 0.9% NaCl.

After 24 hours of wound creation, gently applied an adequate amount of test samples was to cover the wounded area twice a day, in the morning and

afternoon, with cotton swabs for the 11-day duration of the experiment. Wounded areas were observed and measured by a straight ruler on the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, and 11<sup>th</sup> day for all groups. It was essential to assure sterility in order to avoid infection. In case of infection, removed these mice and replace them with others to ensure the number of surveys. The results were evaluated via the percentage of wound contraction, which was calculated by the following formula:  $\%WC = [(WA_0 - WA_n)/WA_0] \times 100$ . In which, WC was the wound contraction,  $WA_0$  was the wound area on day 0, and  $WA_n$  was the wound area on day n.

#### ***Histopathological examination***

Skin tissue specimens after wound formation were collected after 11 days of treatment and transferred into 10% Neutral Buffered Formalin, processed frequently, and embedded with paraffin. 3  $\mu$ m thick sections were prepared and stained with hematoxylin and eosin (H&E) dye, the stained sections were considered and photographed under a microscope (Eclipse TS2R-FL, Nikon, China). The results were observed and further discussed to evaluate the wound-healing effects of the potential preparation.

#### ***Acute dermal irritation/corrosion study***

An acute dermal irritation/corrosion study was carried out according to the guidance of the Ministry of Health, Vietnam (Decision No. 3113/1999/QD-BYT, October 11, 1999)<sup>18</sup>. The method is based on how the rabbit's skin reacts to the reagent compared to the other skin areas without the reagent. Three rabbits with normal, healthy and uniform skin textures were used. The rabbits were shaved from the back evenly to the sides of the spine (about 10 cm  $\times$  15 cm) which represented 10% of the body surface and their skin was washed with 0.9% NaCl 24 hours before the experiment. They received three non-irritating patches of 2.5 cm  $\times$  2.5 cm of the appropriate thickness, each with 0.5 or 0.5 mL dose of prepared test or control samples per rabbit, chloroform and 0.9% NaCl were used as the positive and negative control, respectively. The patches were fixed with nonirritating tapes for at least 4 h. The gauze and tape were then removed, and the remaining reagents were washed with 0.9% NaCl. The rabbits were observed and scored for an adverse skin reaction at 24, 48 and 72 hours after removing the test piece, compared with the untreated areas. Rabbit's skin reactions were evaluated at the levels

of erythema and oedema, assessed and calculated according to the following formula:

$$\text{Dermal Irritation Score (DIS)} = \frac{\text{Value (erythema + oedema)}}{\text{No. of animals No. of observations}}$$

Irritation score with the levels specified into ranges as  $0 < \text{DIS} < 0.5$  indicated non-irritation,  $0.5 \leq \text{DIS} < 2.0$  indicated slight irritation,  $2.0 \leq \text{DIS} < 5.0$  indicated moderate irritation, and  $5.0 \leq \text{DIS} \leq 8.0$  indicated severe irritation<sup>19,20</sup>.

#### ***In vitro antioxidant activity assays***

##### ***DPPH assay***

DPPH free radical scavenging assay was used to evaluate the antioxidant capacity of the potential preparation from Noni leaf extract based on a previously described method<sup>3</sup>. Briefly, a 4 mL reaction mixture in methanol containing 0.5 mL of varying concentrations of the extract and 0.5 mL of 0.6 mM DPPH reagent was kept in the dark at room temperature for 30 minutes. Absorbance was measured at 515 nm and all tests were performed in triplicate. Ascorbic acid was used as a positive control. The results were expressed as  $IC_{50}$  values for each sample.

##### ***ABTS assay***

The ABTS assay was also applied to investigate the antioxidant ability of the potential preparations from Noni leaf extract, the process was carried out according to the following description<sup>21</sup>: ABTS solution was prepared by adding a 7 mM ABTS solution to a 2.4 mM potassium persulfate solution of equal volume and then incubating the solution. Fluid in the dark for 16 hours at room temperature. The solution is then diluted by mixing 1 mL of ABTS solution with 50 mL of methanol to obtain an absorbance of  $0.706 \pm 0.01$  units at 734 nm using a spectrophotometer. Used this solution for testing. About 40  $\mu$ L of the test sample at various concentrations or ascorbic acid (positive control) was mixed with 1160  $\mu$ L of ABTS solution (1: 29 v/v), measuring the absorbance at 734 nm after 6 minutes at room temperature. All tests were done in triplicate and an average of each sample was calculated. The results were expressed as an  $IC_{50}$  value for each sample from the proportion of the radical quenching activity.

The percentage of the radical scavenging activity (DPPH/ABTS) was calculated by the formula:

$$\% \text{ scavenging effect} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where  $\text{Abs}_{\text{control}}$  and  $\text{Abs}_{\text{sample}}$  are the absorbances of the blank (without test extract) and the sample (with test extract), respectively.

#### Qualitative determination of flavonoids

##### Chemical reactions

The solution of the potential preparation from Noni leaf extract was tested for the presence of flavonoids by the following chemical reactions: (1) Alkaline reagent test: Test solution reacts with 10% w/v NaOH solution, the reaction solution increases yellow colour compared to the original solution; (2) Ferric chloride test: Test solution reacts with 5% w/v  $\text{FeCl}_3$  solution, the reaction solution is a nut-brown colour; (3) Lead acetate solution test: Test solution reacts with 10% w/v  $\text{Pb}(\text{CH}_3\text{COO})_2$  solution, the reaction solution shows a white precipitate; and (4) Shinoda's test: Test solution reacts with magnesium ribbon and concentrated HCl, the reaction solution is the reddish-pink colour after a few minutes.

##### Thin layer chromatography (TLC) analysis

The stock solutions of the potential preparation from Noni leaf extract and rutin were prepared. Ascending TLC analysis was performed using Merck aluminium-backed plates, silica gel 60  $F_{254}$  as a stationary phase and four solvent systems with differing polarities including *n*-butanol: Acetic acid: Distilled water (7: 1: 2, v/v/v) and Ethyl acetate: Formic acid: Acetic acid: Distilled water (34: 3.5: 1.5: 7, v/v/v/v), used as a mobile phase to analyse the test solution. The solvent system was saturated for 30 minutes before deploying chromatography. A small number of samples was detected at about 0.5 cm from the bottom of the TLC plate and then developed for 15 min in the chamber. Subsequently, the plate was taken out of the chamber and air-dried. Visible bands were viewed under UV light at 254 nm. Then, 5% iron (III) chloride reagent was sprayed on the TLC plate in order to visualize rutin<sup>3</sup>. The coloured bands that emerged were marked and the retention factor ( $R_f$ ) was calculated using the following formula:

$$R_f = \frac{\text{Distance traveled by a compound}}{\text{Distance traveled by solvent front}}$$

#### Quantification of total flavonoid content of the potential preparation

##### Spectrophotometric determination using rutin as the standard

Quantification of total flavonoid content followed the previously described protocol with minor modifications<sup>3</sup>. The total flavonoid content was determined photometrically when reacting with an aluminium chloride ( $\text{AlCl}_3$ ) reagent using rutin as the standard. The standard rutin stock solution was obtained by dissolving 1.0 mg of rutin in 10 mL of methanol, then diluted consecutively with methanol to construct the standard calibration curve. The reaction mixture consisted of a 200  $\mu\text{L}$  diluted test sample from the potential preparation or standard rutin solution, and 200  $\mu\text{L}$  2% aluminium chloride ( $\text{AlCl}_3$ ), then adjusted to 2 mL with methanol. Afterwards, mixed well and incubated the mixture for 15 min at room temperature. The absorbance was measured using a spectrophotometer at the maximum absorption wavelength of the rutin standard (431 nm). The measurements were done in triplicates. The total flavonoid content was calculated from the rutin calibration curve equation ( $Y = 0.0053x - 0.0774$ ,  $R^2 = 0.9990$ ), and the results were expressed as  $\mu\text{g}$  rutin equivalents/g dry weight.

##### Determination of rutin by high performance liquid chromatography (HPLC)

The rutin content was estimated by the HPLC method using rutin as a standard. Prepared the standard stock solution by weighing exactly 10 mg of the rutin standard, then dissolved and adjusted to 5 mL with methanol. From there, continue diluted to a standard series of concentrations (50 - 400  $\mu\text{g}/\text{mL}$ ), which were then filtered through a 0.45  $\mu\text{m}$  membrane filter before HPLC analysis. For the test sample, accurately weighed 5 g of the potential preparation into a filter paper bag, ultrasonically extracted with 20 mL *n*-hexane and then discarded the *n*-hexane solution was to remove impurities, repeated 5 times, 30 min each time. Further extracted the sample with 30 mL methanol 5 times, the methanol extracted solution was collected and evaporated under reduced pressure to gain dry extract. Afterwards, dissolved and adjusted to 5 mL with methanol, continuously filtered through a 0.45  $\mu\text{m}$  pore size filter and then degassed before being injected into the HPLC system for quantitative analysis. The analysis was carried out in triplicate to calculate the average mean.

*HPLC conditions:* The chromatographic separation was carried out on a Venusil XBP C18 column

(4.6×250 mm, 5 µm particle size) by eluting at a flow rate of 0.7 mL/min using a gradient with acetonitrile (A) and 0.1% formic acid (B). The mobile phase followed the gradient elution program: 1–30% A at 0–30 min, 30–80% A at 30–40 min, 80% A at 40–45 min, 80–30% A at 45–50 min, 30–1% A at 50–60 min, and 1% A at 60–65 min. The mobile phase composition returned to the initial condition for 10 min and balanced for 5 minutes before the injection of another sample. The injection volume was 5 µL and the analytical wavelength of rutin was 254 nm. The quantification was carried out by integrating the chromatographic peak, which was validated by comparing the test sample retention time and UV spectra to the reference standard - rutin. The rutin content was calculated and expressed as a percentage of rutin through the regression equation of

$Y = 41855x + 412100$ ,  $R^2 = 0.9966$ , where  $y$  and  $x$  were peak area and compound concentration.

#### Data analysis

The results were expressed as mean±SEM (Standard error of the mean). The data were analyzed using paired  $t$ -test and One-Way ANOVA to compare statistics. All the data analyses were carried out by Graph-Pad Prism (version 8, Inc., La Jolla, CA, USA) and MS Excel 2016 software.  $P < 0.05$  was considered statistically significant.

### Results

#### Preparation and formulation of *M. citrifolia* leaf extract

The finished products of cream, ointment and powder preparation after formulating were displayed in Fig. 1. Through observation, the cream 1%

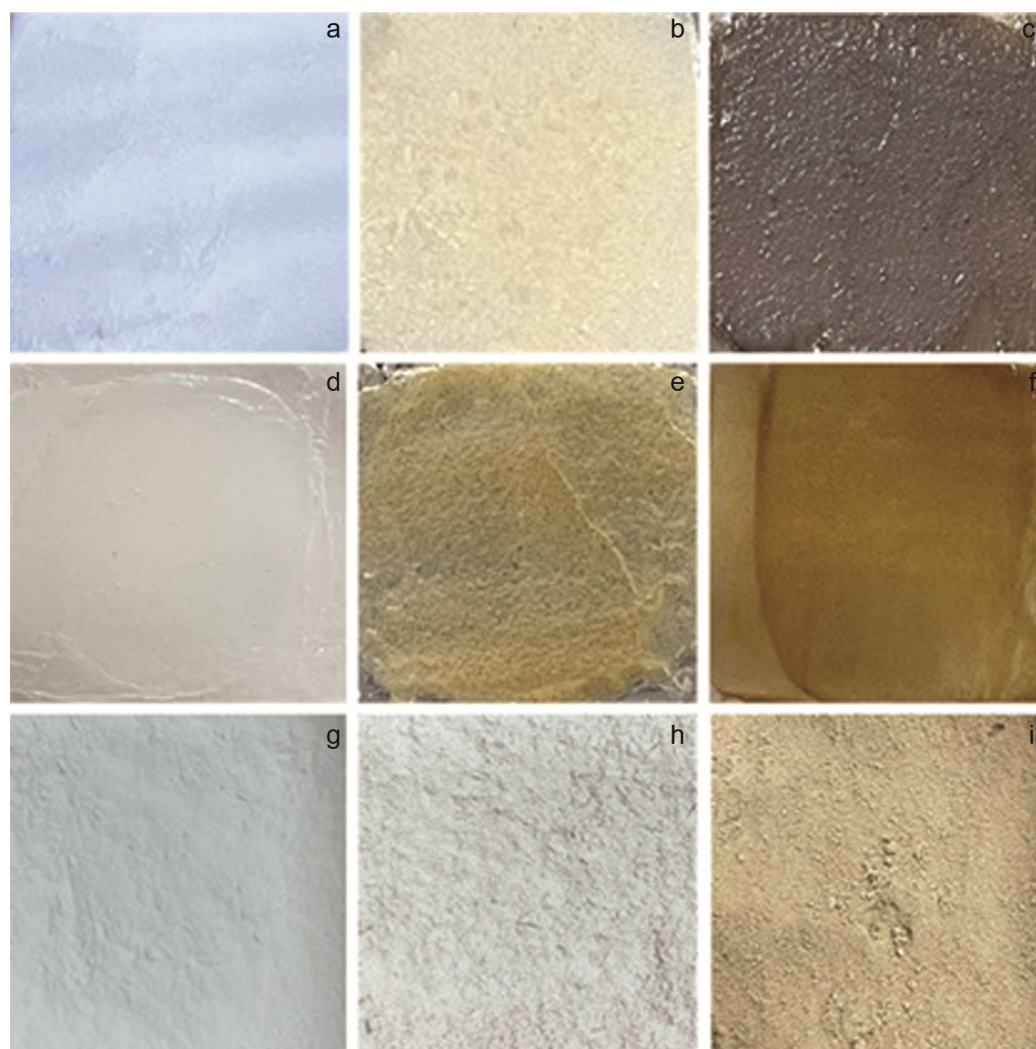


Fig. 1 — Formula preparation of *M. citrifolia* leaf extract. Cream base with extract at, a) 0%; b) 1%; and c) 5% concentration; ointment base with extract at, d) 0%; e) 1%; and f) 5% concentration; powder base with extract at, g) 0%; h) 1%; and i) 5% concentration.

standardized extract formula appeared to be creamy and had a smoother and softer texture in comparison with cream 5% and the cream base. Besides, ointment preparations appeared to have a greasy, sticky and thicker form, whereas the ointment 5% extract was likely more of a liquid texture than the ointment 1%, and the Noni leaf extract was slightly separated from

the ointment base. All powdered formulas were in solid form, with a fine and smooth texture.

#### Wound healing activity of the herbal preparations

During the healing period, the wounds were regularly measured on the 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup> and 11<sup>th</sup> day, which showed the evolution of the wound surfaces closure. The results are represented in Fig. 2, which



Fig. 2 — Photographs represent the appearance of excision wound healing during the 11-day study period of the preparations from *M. citrifolia* leaf extract, negative control (NaCl 0.9%), and standard (Gentamicin 0.1%) treated mice.

showed a decline in the wound area over time in all preparations treated groups after 11 days, especially the group treated with cream and ointment. Table 2 displayed wound contraction percentages of the Noni leaf extract cream, ointment and powder at 1 and 5% concentration to compare their healing efficacy. In the beginning, the Noni leaf extract formulas treated groups were observed to cause minor inflammation and scab formation, therefore when measuring, the wound areas were slightly larger in comparison with day 1. However, this phenomenon only lasted a few days and the level gradually decreased, and after the scab fell off, the wounds were in fact healed and degraded in size. On the 7<sup>th</sup> day of the experiment, the wound size in cream 1% and 5% treated mice significantly decreased ( $P < 0.0001$ ) than those of day 1; with wound shrinkage percentages were 45.718% and 43.506%, respectively; and statistically higher than the others. After 11 days, the better contraction was recorded in cream 1% (92.708%) and 5% (94.444%) treated groups, and cream 5% was considerably different ( $P < 0.05$ ) compared to powder 5%, showing the least healing effect with only 65.370%. Therefore, the creams 1% and 5%

were chosen as the potential preparations for further evaluation.

Table 3 showed the wound area measurements of the cream formulations compared to gentamicin (standard) and NaCl 0.9% (negative control). At first, gentamicin and NaCl 0.9% illustrated better healing results with no inflammation responses, compared to groups treated with cream, and their closure percentages experienced a significant increase on day 5 from day 1. However, results demonstrated that both cream 1 and 5% groups showed noteworthy differences ( $P < 0.05$ ) to NaCl 0.9% on day 7. On the last day, these cream groups displays improved and complete skin recovery, statistically better ( $P < 0.05$ ) than the negative control – NaCl. Cream 1 and 5% treated results confirmed no significant differences from each other, reaching 92.708 and 94.444%, respectively, however, they performed greater contraction compared to gentamicin (89.931%) and 0.9% NaCl (76.389%). It can be concluded that both have the same healing ability and cream 1% was chosen for further investigations.

Table 2 — Wound healing effect (% WC) of different *M. citrifolia* leaf extract preparations in excision wound model to screen for the potential formula

Conc. Preparation	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
1% Cream	0	-2.083±2.083	6.829±4.905 <sup>#</sup>	45.718±3.013	73.495±2.513	91.667±2.635
Ointment	0	-16.402±7.259	-1.339±2.783	28.191±6.941 <sup>*</sup>	71.065±2.352	91.336±4.367
Powder	0	-7.143±4.880	2.817±5.718	20.873±5.931 <sup>bb</sup>	50.648±9.616 <sup>*</sup>	77.553±5.666 <sup>b</sup>
5% Cream	0	-15.278±3.829 <sup>*</sup>	2.778±5.659	43.056±3.167	70.486±3.156 <sup>d</sup>	94.444±2.561 <sup>dd</sup>
Ointment	0	-25.265±7.159 <sup>*</sup>	-20.106±5.599 <sup>a</sup>	26.323±5.808 <sup>b</sup>	69.180±4.449	88.955±2.873
Powder	0	-2.778±2.778 <sup>#</sup>	6.019±2.776 <sup>#</sup>	24.329±6.556 <sup>b</sup>	44.722±6.272 <sup>cc</sup>	65.370±8.575 <sup>c</sup>

<sup>\*</sup> $P < 0.05$ , significant difference compared to cream 1%; <sup>#</sup> $P < 0.05$ , a significant difference compared to ointment 5%; <sup>b</sup> $P < 0.01$ , significant difference compared to ointment 5%; <sup>a</sup> $P < 0.05$ , significant difference compared to ointment 1%, cream 5%, powder 1%; <sup>b</sup> $P < 0.05$ , significant difference compared to cream 1% and 5%; <sup>bb</sup> $P < 0.01$ , significant difference compared to cream 1% and 5%; <sup>c</sup> $P < 0.05$ , significant difference compared to cream 1%, ointment 1% and 5%; <sup>cc</sup> $P < 0.01$ , significant difference compared to cream 1%, ointment 1% and 5%; <sup>d</sup> $P < 0.05$ , significant difference compared to powder 5%; <sup>dd</sup> $P < 0.01$ , significant difference compared to powder 5%. n = 6, mean±SEM.

Table 3 — Percentages of wound contraction when treated with potential preparations, compared to control and standard

Group	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
Cream base	0	0.000 ± 7.217	1.042 ± 3.391 <sup>***</sup>	39.120 ± 6.205	62.831 ± 3.829 <sup>@</sup>	88.194 ± 5.933
Cream 1%	0	-2.083 ± 2.083 <sup>S</sup>	6.829 ± 4.905 <sup>**</sup>	45.718 ± 3.013 <sup>#</sup>	73.495 ± 2.513 <sup>###</sup>	91.667 ± 2.635 <sup>##</sup>
Cream 5%	0	-15.278 ± 3.829 <sup>***</sup>	2.778 ± 5.659 <sup>**</sup>	43.056 ± 3.167 <sup>#</sup>	70.486 ± 3.156 <sup>#</sup>	94.444 ± 2.561 <sup>##</sup>
Gentamicin 0.1%	0	9.474 ± 1.485 <sup>@@</sup>	24.735 ± 2.610	34.276 ± 4.938	70.866 ± 5.968	89.931 ± 4.640 <sup>#</sup>
NaCl 0.9%	0	10.317 ± 3.932 <sup>@</sup>	25.595 ± 2.415	34.524 ± 1.920	57.341 ± 3.857	76.389 ± 3.510

<sup>S</sup> $P < 0.05$ , significant difference compared to cream 5%; <sup>\*\*</sup> $P < 0.01$ , significant difference compared to gentamicin and NaCl 0.9%; <sup>\*\*\*</sup> $P < 0.001$ , significant difference compared to gentamicin and NaCl 0.9%; <sup>@</sup> $P < 0.05$ , significant difference compared to cream 1%; <sup>@@</sup> $P < 0.01$ , significant difference compared to cream 1%; <sup>#</sup> $P < 0.05$ , significant difference compared to NaCl 0.9%; <sup>##</sup> $P < 0.01$ , significant difference compared to NaCl 0.9%. n = 6, mean±SEM.

### Histopathological study

The histological sections of the excised wound after 11-day wounding of cream preparations treated groups, compared with before and after treated with positive (Gentamicin) and negative (NaCl 0.9%) controls were examined in Fig. 3. The skins were stained and analyzed the epidermis and dermis layer. The epidermis is divided into four layers: stratum corneum, granular layer, spinous layer, and basal layer; and the dermis layer was where the inflammatory cells penetration could be observed<sup>22</sup>. Normal skin tissue consisted of an epidermal layer with many spinous cells and basal cells, a keratinized surface with a thin stratum corneum. The dermis was composed of scattered fibroblasts. Skin structures in the cream 1% and Gentamycin treated groups were found to be within normal epithelization, similar to the normal skin. They showed an increased number of keratinocytes, granular and spinous cells compared to the before-treatment group, fibroblast proliferation and angiogenesis presented, and fewer penetrated inflammatory cells and neutrophils than the other groups, except for the normal skin one. In contrast, the histology of excision biopsy of wounded skin in the cream base applied group showed a thick

stratum corneum, with epidermolysis, fibre decomposition and subepidermal haemorrhage phenomena appeared, many infiltrated immune cells and strong fibroblast proliferation presented. In the negative control group, results indicated a large amount of epidermal and immune cells, fibroblasts, and occurrence of epidermolysis, oedema, diffuse congestion and profuse haemorrhage. The histological study also showed significant neovascularisation in all wounded mice.

### Acute dermal irritation test

Fig. 4 displayed the rabbits' skin appearance at different times of evaluation, applied with NaCl 0.9% (negative control), Chloroform (positive control) and the chosen Noni leaf extract preparation – Cream 1% in the acute dermal irritation/corrosion study. The 0.9% NaCl treated skin areas showed no signs of erythema, oedema, and no abnormalities during the test. The skins where chloroform patches were placed had visible redness from 1-hour interval after removing the patch and cleaning the skin, with an erythema level from 1 to 3, the redness persisted after 24, 48, and 72 hours. Results also indicated the skin area

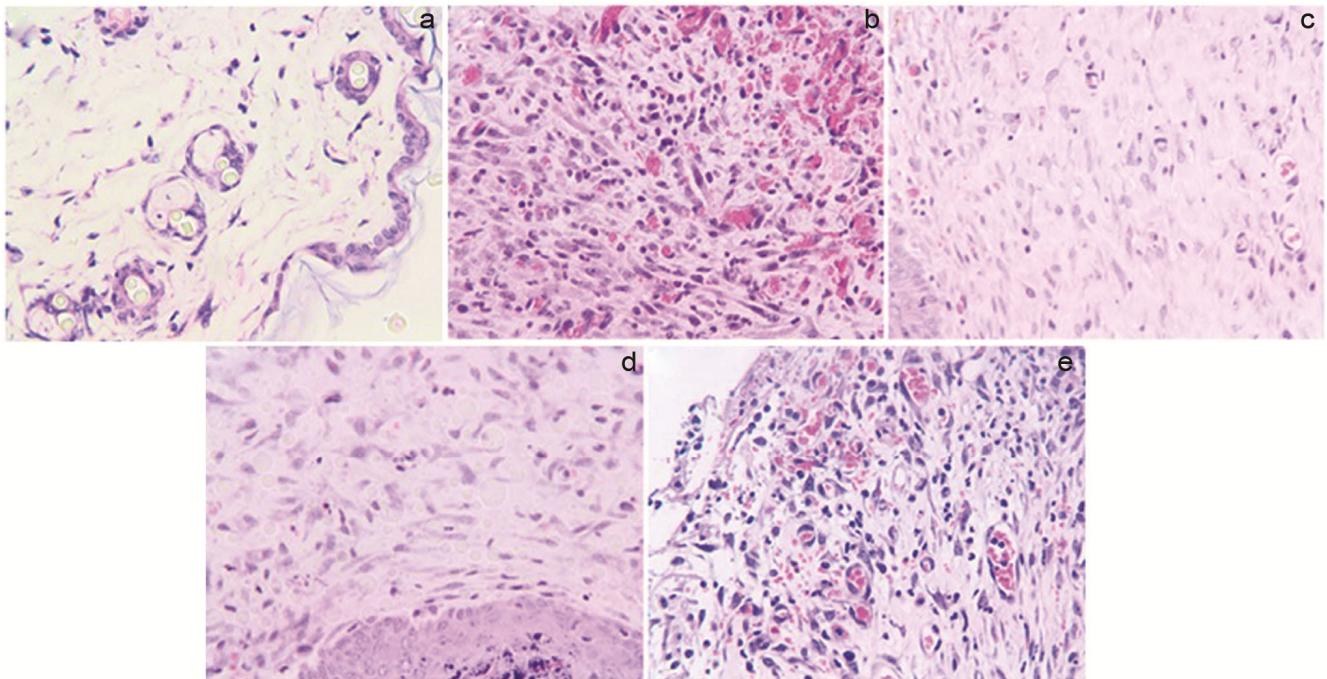


Fig. 3 — Histological evaluation of skin sections stained with hematoxylin and eosin (40×magnification). a) Normal skin features before treatment; b) cream base treatment, showing more inflammatory cells and haemorrhage appeared; c) skin tissue with normal regenerative proliferation observed with cream 1%; d) positive control with normal skin features; and e) negative control with a moderate level of subcutaneous structural inflammation caused by the penetration of inflammatory cells and neutrophils.



Fig. 4 — Photographs display the rabbits' skin appearance after different time intervals (24, 48, and 72 hours). The skin area where, a) NaCl 0.9%; b) chloroform; and c) cream 1% patches were placed.

applied directly with cream 1% was slightly red 24 hours after cleansing, following that, the skin returned to normal condition as the untreated skin. The Dermal Irritation Score of the test sample was equal to “0.22” compared to the positive and negative control, “1.00” and “0,” respectively. Therefore, it can be concluded that cream 1% from Noni leaf extract caused negligible dermal irritation in rabbits.

#### **Antioxidant activity of potential preparation from *M. citrifolia* leaf extract**

During the wound healing process, the antioxidant barrier also plays an important role due to the continuous increase in cell activity. At low levels,

oxidants are beneficial for protection from bacterial colonization; intracellular signal transduction and angiogenesis. However, excessive oxidants reduce its beneficial effects because it is highly reactive and can lead to tissue damage, hindering wound healing<sup>23</sup>. Thus, the antioxidant activity of Noni leaf cream 1% was evaluated using *in vitro* assays. The results showed that the cream preparation containing 1% Noni leaf extract had a good antioxidant effect via DPPH and ABTS free radical scavenging ability (Fig. 5). DPPH free radical quenching activity of cream was lower than that of ascorbic acid with IC<sub>50</sub> values of  $30.20 \pm 1.07$  and  $4.60 \pm 0.07$   $\mu\text{g/mL}$ , respectively, while cream had the ability to neutralize

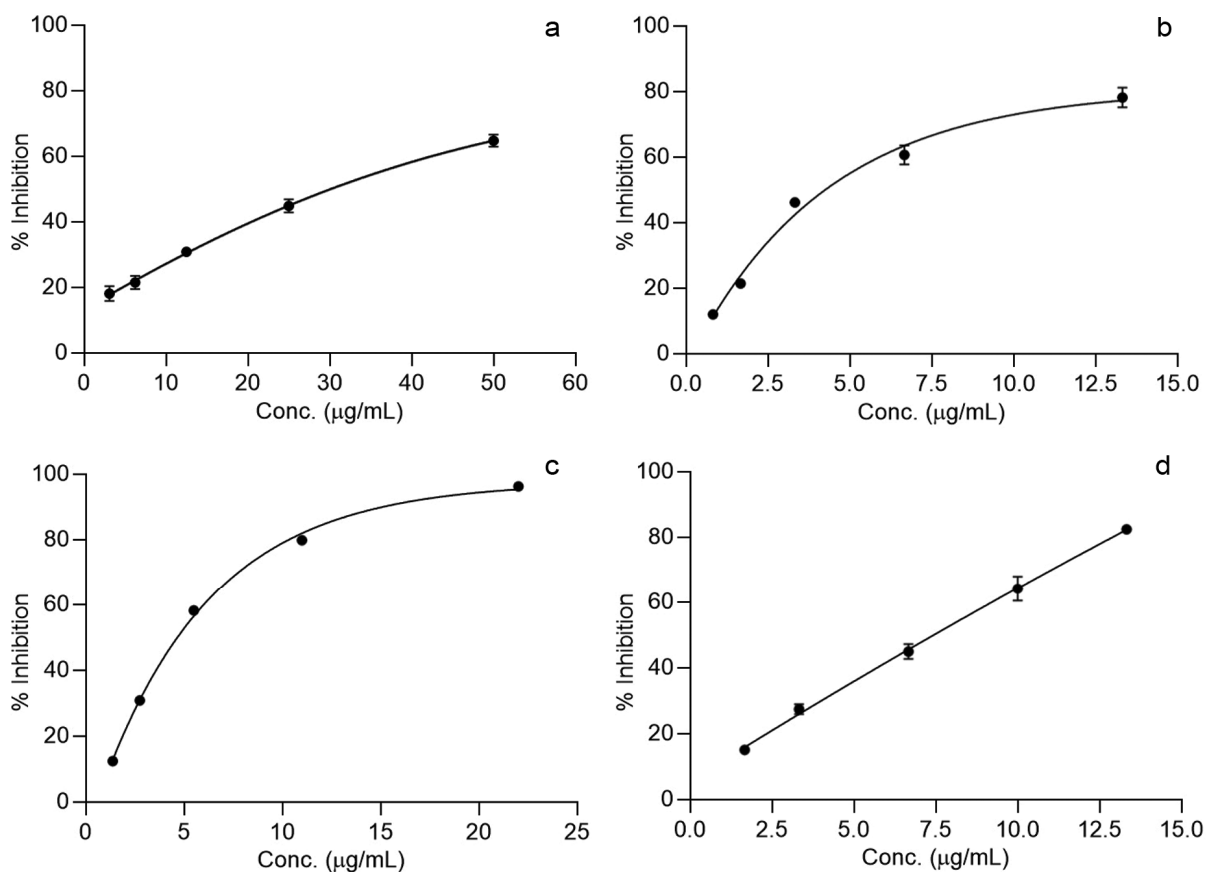


Fig. 5 — Antioxidant effect of cream 1% from Noni leaf extract by inhibition of DPPH and ABTS. Per cent inhibition of cream against, a) DPPH; and b) ABTS. Per cent inhibition of ascorbic acid against, c) DPPH; and d) ABTS.

ABTS free radicals 1.62 times better than ascorbic acid with  $IC_{50}$  values of  $4.33 \pm 0.20$  and  $7.02 \pm 0.38$  µg/mL, sequentially.

#### Qualitative determination and quantification of flavonoids

In our previous study, flavonoids were shown to be the main group of compounds in Noni leaf extract and rutin was selected as a marker. In the present study, flavonoids were again identified in cream containing 1% Noni leaf extract through qualitative and quantification methods.

The results of chemical reactions illustrated the solution of cream containing 1% Noni leaf extract gave positive results with all four reagents specific for flavonoids (NaOH 10%,  $FeCl_3$  5%,  $Pb(CH_3COO)_2$  10% v/v solutions, cyanidin reagent), which showed that Noni leaf cream contains flavonoids. At the same time, the TLC profiling results indicated the presence of rutin within the Noni leaf cream with the colour and  $R_f$  value of the band obtained from cream equivalent to rutin standard, 0.35 (Ethyl acetate: Formic acid: Acetic acid: Distilled water, 34: 3.5: 1.5:

7, v/v) and 0.53 (*n*-butanol: Acetic acid: Distilled water, 7: 1: 2, v/v).

The total flavonoid content of Noni leaf cream was determined by a spectrophotometric method using rutin as the standard, which got  $27.50 \pm 0.92$  µg rutin equivalents/g of dry weight. The results of rutin content determined by HPLC analysis using rutin as the standard was performed. The retention time of the rutin reference standard and test sample (Noni leaf cream 1%) peaks were detected at 30.543 and 30.463, respectively. The representative chromatograms observed in Fig. 6 illustrated that the Noni leaf cream sample had a peak with similar retention times to that of the rutin standard. From the results of peak areas with a retention time corresponding to the rutin of the cream sample, rutin content (%) in the cream 1% sample was  $0.0047\% \pm 0.00008$ .

#### Discussion

*Morinda citrifolia* L. (Noni) leaves have been used as a traditional herb for wound healing activity<sup>13</sup>, and have been proven to have antioxidant, antimicrobial,

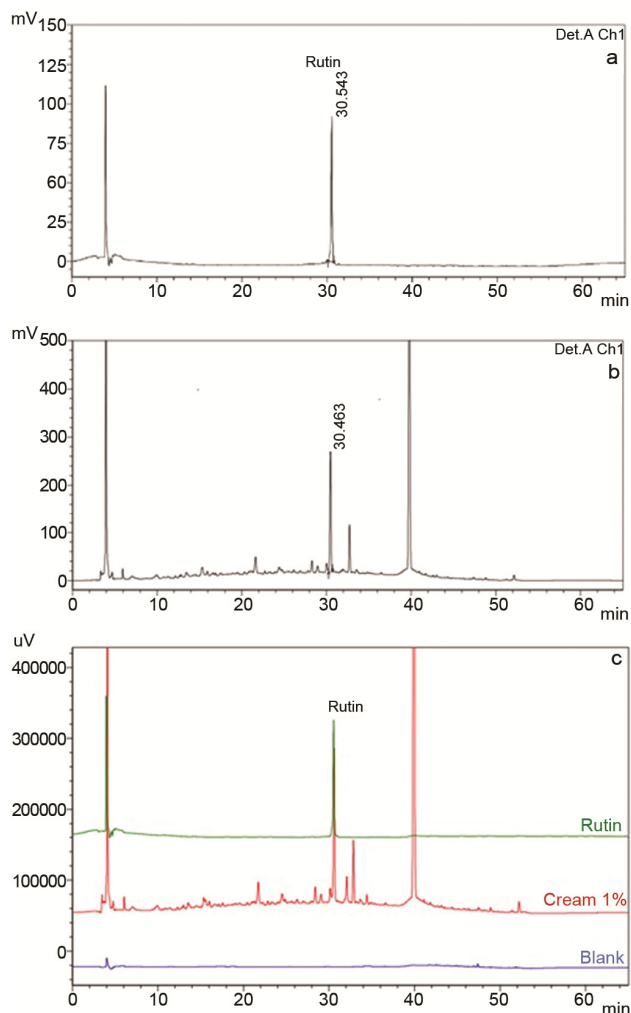


Fig. 6 — HPLC chromatogram for rutin quantification at 254 nm of cream 1% from Noni leaf extract. Chromatographic analysis of, a) Rutin (standard); b) cream 1%; and c) comparison of blank, test sample and standard.

and anti-inflammatory bioactivities, as well as promoting wound healing acceleration<sup>8,15</sup>. Inheriting from our previous research at the Research Center of Ginseng and Medicinal Materials Ho Chi Minh City<sup>3</sup>, the wound healing efficacy of some preparation types from *M. citrifolia* leaf extract was investigated in this study to choose the potential one. An appropriate choice of administration route is critical for maximizing their bioavailability and efficacy, more importantly, for developing commercial products in the future. Therefore, it is essential to develop one that applies directly on the skin instead of using crude extracts, that guarantee a similar desired quality as its original herb extract. Topically applied formulations exist in many forms, however, this study focused on 3 product types: cream, ointment, and powder. Cream

and ointment are the most commonly used semisolid dosage types, as creams are easily absorbed in the skin and ointment doesn't evaporate off the skin easily, ensuring maximum product absorption. The powder, on the other hand, has been proven for its good absorbance when apply to open wounds and its stability under various storage conditions<sup>24</sup>. As a result, the final preparations after formulating all showed a fine texture and demonstrated good absorbance when applied to the wounds.

In order to evaluate the wound healing effectiveness of Noni leaf extract preparations after daily application, a wound excision model on mice was carried out to compare each type, through the percentage of contraction. The results indicated that Noni leaf cream 1% and 5% showed better healing properties with almost completely closed wounds after 11 days. This assay concluded that the Noni leaf creams were the most appropriate topical preparations for transporting the active ingredients that support healing into the injured areas to maximize their effectiveness, which can be explained due to their good absorbance to the skin. Therefore, preparations in cream form were chosen as the potential formulations for further evaluation, as their wound healing activities were also remarkably better in comparison with the negative control on day 11 post-wounding. They even possessed greater wound contraction percentages than the standard gentamicin, however, there were no significant differences between them. Although in the beginning, these creams-treated groups triggered negligible inflammation, their level reduced in the following days. It caused larger scabs, and after scab dropping, the wound sizes continued to reduce more rapidly than the control groups, which can be easily observed from day 7. Besides, creams at different concentrations confirmed similar healing abilities, so the preparation that showed better results at lower concentrations is more beneficial in terms of product formulation, safety and economic benefits. The results of this study were similar to those reported in previous research that evaluated the wound healing acceleration of *M. citrifolia* leaf extracts<sup>3,14,25</sup>, in which the increase in wound closure can be explained by the bioactivities of Noni leaves that contribute to the healing effect.

Close observation and analysis of healed tissue histological sections after the wound excision could help to confirm the results and provide more

information about the angiogenesis, epithelialization, proliferation of fibroblasts, and epidermis layer including stratum corneum, granular and Malpighian layer. Earlier researchers stated that complex interactions between a large number of cell types, including endothelial and immune cells, keratinocytes, fibroblasts, and others that are involved in the 3-stage healing process are crucial for successful wound repair and closure<sup>22</sup>. Histopathological samples evidenced that the recovered wound sites after 11 days demonstrated an increase in the number of keratinocytes, granular and spinous cells in the epidermis layer, as well as the enhanced neovascularisation, fibroblast proliferation, with the amount of infiltrated inflammatory cells and neutrophils in comparison with pre-injury skin tissue. Moreover, skin areas of cream 1% and gentamicin-treated mice were within the normal range, similar to the before treatment one, and were analyzed with fewer fibroblasts and immune cells than in the cream base and the negative control groups, which showed the opposite results of more inflammatory cells and fibroblasts. Studies have suggested that the shrinkage of the wound area was associated with the movement of fibroblasts to injured tissues<sup>3</sup>, which could be understood as the plant-induced fibroblast proliferation and differentiated into myofibroblasts, then their high contractile activity and the movement of epithelial cells generated would result in wound closure<sup>26</sup>. However, moderate fibroblast proliferation was presented in the cream-treated wound site, fewer than in the others. This could be reasonably explained by the work of previous research proving Noni leaves had better acceleration in healing, indicating that the number of fibroblasts in the treated group had reduced earlier than in the control group<sup>25</sup>. Besides, the epithelialization time was shortened as a result of these factors on wound healing, and the wounds were almost fully healed within 11 days.

In order to introduce a new drug in topical form into the marketplace, an approved testing procedure for producing scientifically reliable data on the irritation is critical to validate its safety before employing it in clinical treatments. Former studies reported that some plant extract active ingredients may possess toxicological potential, and topical adverse effects of natural products were recorded as allergic, irritant contact dermatitis and phytophotodermatitis could appear<sup>27</sup>. Since animal skin is quite sensitive to most chemicals, all new formulations must be tested to

check for any occurrence of dermal responses<sup>28</sup>. Hyperemia of superficial capillaries causes erythema, which is redness of the skin or mucous membranes while oedema is a type of swelling produced by excess fluid in the body's tissues<sup>27</sup>. Therefore, this thesis project performed the acute dermal irritation study of the most potential preparation from *M. citrifolia* leaf extract on rabbits, based on the Ministry of Health guidelines. The results revealed that only minor redness was recorded after 24 hours of removing the cream 1% patch and cleansing, then returned to normal condition in the following hours. Hence, no severe erythema, oedema or any skin lesion was observed after the application. Based on the findings, Noni leaf cream 1% caused insignificant dermal irritation and seemed to be safe for usage.

*M. citrifolia* leaf has been proven to increase wound contraction rate, and reduce the time of granulation tissue formation that results in the healing of wounds<sup>29</sup>, whereas its chemical components including flavonoids, terpenoids and iridoid glycosides possess wound healing property<sup>15</sup>. Flavonoids are known to promote healing activity mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound shrinkage and increase epithelialization rates<sup>13</sup>. Rutin, one of the most common pharmacologically active ingredients, was proved to be the main flavonoid isolated from *M. citrifolia* leaves, and in recent publications on Noni leaves, scientists have used rutin as a biomarker to identify the presence of flavonoids or specifically, flavonol glycosides<sup>30-32</sup>. Rutin was proven to promote wound closure by inhibiting the production of inflammatory cells, reducing oxidative stress in the injured area, promoting the formation of neo-epithelium and thicker granulation, as well as supporting tissue regeneration<sup>33</sup>. Besides, in recent days, plant extracts containing flavonoids have been widely used in dermatological preparations, primarily due to skin penetrating ability, specifically, rutin can permeate through the epidermis and dermis layers<sup>34</sup>. In this study, flavonoids and rutin have been detected in Noni leaf cream, it could probably be one of the factors contributing greatly to the wound healing efficacy of a cream containing *M. citrifolia* leaf extract.

## Conclusion

The chosen potential preparation was cream with a 1% concentration of *M. citrifolia* leaf extract, showing

the most effective ability in wound healing acceleration and epidermal and skin tissue regeneration. Noni leaf cream 1% did not cause severe irritation when administered on rabbit skin. The cream also had a good *in vitro* antioxidant effect. The content of total flavonoids and rutin compounds in the potential topical formula indicated its contribution to wound healing activity. This study has created the promising potential for further product development on *M. citrifolia* leaf extract formulations for transdermal drug delivery in wound treatment.

### Acknowledgement

The authors express the gratitude to the National Institute of Medicinal Materials, Vietnam for the financial support under grant number 06/2020/HD-NVTX-TTS.

### Conflicts of interest

All the authors of this manuscript declare that there is no conflict of interest.

### References

- Tartarini D and Mele E, Adult stem cell therapies for wound healing: Biomaterials and computational models, *Front Bioeng Biotechnol*, 2016, **3**, 206.
- Wang P H, Huang B, Horng H C, Yeh C and Chen S A, Wound healing, *J Chin Med Assoc*, 2017, **81**(2), 94-101.
- Ly H T, Nguyen P M T, Nguyen T K O, Bui T P Q, Ke X, *et al.*, Phytochemical analysis and wound-healing activity of Noni (*Morinda citrifolia*) leaf extract, *J Herbs Spices Med Plants*, 2020, **26**(4), 1-15.
- Reinke J M and Sorg H, Wound repair and regeneration, *Eur Surg Res*, 2012, **49**(1), 35-43.
- Pazyar N, Yaghoobi R, Rafiee E, Mehrabian A and Feily A, Skin wound healing and phytomedicine: A review, *Skin Pharmacol Physiol*, 2014, **27**(6), 303-310.
- Medellín-Luna M, Castañeda-Delgado J E, Martínez-Balderas V and Cervantes-Villagrana A, Medicinal plant extracts and their use as wound closure inducing agents, *J Med Food*, 2019, **22**(5), 435-443.
- Wang M Y, West B, Jensen C J, Nowicki D, Su C, *et al.*, *Morinda citrifolia* (Noni): A literature review and recent advances in Noni research 1, *Acta Pharmacol Sin*, 2003, **23**(12), 1127-1141.
- Almeida É, Oliveira D and Hotza D, Properties and applications of *Morinda citrifolia* (Noni): A review, *Compr Rev Food Sci Food Saf*, 2019, **18**(4), 883-909.
- Inada A C, Figueiredo P S, Santos-Eichler R A D, Freitas K C, Hiane P A, *et al.*, *Morinda citrifolia* Linn. (Noni) and its potential in obesity-related metabolic dysfunction, *Nutrients*, 2017, **9**(6), 540.
- Assi R, Darwis Y, Abdulbaqi I M, Khan A A, Vuanghao L, *et al.*, *Morinda citrifolia* (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials, *Arab J Chem*, 2017, **10**(5), 691-707.
- Lohani M, Majrashi M, Govindarajulu M, Patel M, Ramesh S, *et al.*, Immunomodulatory actions of a Polynesian herb Noni (*Morinda citrifolia*) and its clinical applications, *Complement Ther Med*, 2019, **47**, 102206.
- Torres M, Magalhães I, Mondêgo-Oliveira R, de Sá J C, Rocha A L, *et al.*, One plant, many uses: A review of the pharmacological applications of *Morinda citrifolia*, *Phytother Res*, 2017, **31**(7), 971-979.
- Nayak B S, Sandiford S and Maxwell A, Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf, *Evid-based Complement Altern Med*, 2009, **6**(3), 251-256.
- Rasal V, Sinnathambi A, Ashok P and Yeshmaina S, Wound healing and antioxidant activities of *Morinda citrifolia* leaf extract in rats, *Iran J Pharmacol Ther*, 2008, **7**(1), 49-52.
- Rezeki S, Sunnati S and Vidirachmilla N, The effect of Noni leaves extract (*Morinda citrifolia* L.) on wound healing percentage of traumatic ulcer in oral mucosa of Wistar rats (*Rattus norvegicus*) by *in vivo*, *Biomed Pharmacol J*, 2017, **10**(4), 1735-1740.
- Semwal R, Joshi S K, Semwal R B and Semwal K D, Health benefits and limitations of rutin - A natural flavonoid with high nutraceutical value, *Phytochem Lett*, 2021, **46**, 119-128.
- Chen L Y, Huang C N, Liao C K, Chang H M, Kuan Y H, *et al.*, Effects of Rutin on wound healing in hyperglycemic rats, *Antioxidants*, 2020, **9**(11), 1122.
- Ly H T, Nguyen T D T, Phung T T H, Tran B H and Le V M, Optimizing the extraction conditions of Noni leaves (*Morinda citrifolia* L.) by response surface method, *Vietnam J Sci Technol Eng*, 2019, **61**(9), 22-28.
- Ministry of Health Vietnam, Test guideline: Acute dermal irritation/corrosion test (decision No. 3113/1999/QD-BYT, October 11, 1999), Vietnam.
- Draize J H, Woodard G and Calvery H O, Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, *J Pharmacol Exp Ther*, 1944, **82**(3), 377-390.
- Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, *et al.*, Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays, *J Nutr*, 2003, **133**(9), 2812-2819.
- Zomer H D and Trentin A G, Skin wound healing in humans and mice: Challenges in translational research, *J Dermatol Sci*, 2018, **90**(1), 3-12.
- Guo S D and DiPietro L A, Factors affecting wound healing, *J Dent Res*, 2010, **89**(3), 219-229.
- Chang R K, Raw A, Lionberger R and Yu L, Generic development of topical dermatologic products: Formulation development, process development, and testing of topical dermatologic products, *AAPS J*, 2013, **15**(1), 41-52.
- Sabirin I P and Yuslianti E R, Benefits of ethanol based Noni leaf (*Morinda citrifolia* L.) extract on oral mucosal wound healing by examination of fibroblast cells, *J Dent Indones*, 2016, **23**(3), 59-63.
- Yassine A K, Hemida H, Mokhtar B, Karim A, Hadjer S, *et al.*, A topical ointment formulation containing leaves' powder of *Lawsonia inermis* accelerate excision wound healing in Wistar rats, *Vet World*, 2020, **13**(7), 1280-1287.

- 27 Gatne M M, Tambe K, Adarsh and Ravikanth K, Acute dermal irritation study of polyherbal gel mastilep in rabbits, *Int J Pharm Sci Res*, 2015, **12**(6), 3473-3476.
- 28 Wang J, Li Z, Sun F, Tang S, Zhang S, *et al.*, Evaluation of dermal irritation and skin sensitization due to vitacoxib, *Toxicol Rep*, 2017, **4**, 287-290.
- 29 Singh B and Sharma R, Indian *Morinda* species: A review, *Phytother Res*, 2019, **34**(5), 924-1007.
- 30 Gu H, Boonanantanasarn K, Kang M, Kim I, Woo K M, *et al.*, *Morinda citrifolia* leaf extract enhances osteogenic differentiation through activation of Wnt2<sup>2</sup>-Catenin signaling, *J Med Food*, 2018, **21**(1), 57-69.
- 31 Wattanathorn J, Thipkaew C, Thukham-mee W, Muchimapura S, Wannanon P, *et al.*, *Morinda citrifolia* L. leaf extract protects against cerebral ischemia and osteoporosis in an *in vivo* experimental model of menopause, *Oxid Med Cell Longev*, 2018, **2018**, 1039364.
- 32 Wigati D, Anwar K, Sudarsono and Nugroho A E, Hypotensive activity of ethanolic extracts of *Morinda citrifolia* L. leaves and fruit in Dexamethasone-induced hypertensive rat, *J Evid Based Complement Altern Med*, 2017, **22**(1), 107-113.
- 33 Ganeshpurkar A and Saluja A K, The pharmacological potential of rutin, *Saudi Pharm J*, 2016, **25**(2), 149-164.
- 34 Boligon A, Jesus R, Piana M, Brum T F d, Cruz R C d, *et al.*, Development and validation of an HPLC-DAD analysis for flavonoids in the gel of *Scutia buxifolia*, *J Chromatogr Sci*, 2015, **53**(9), 1557-1561.