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Wound Healing Activity of Nanoemulgel Containing *Artocarpus lakoocha* Roxb. Extract on Burns Model in Rat

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Abstract

BACKGROUND: The content of secondary metabolites in mobe leaves has the potential to be used in wound healing. Artocarpine, one of the secondary metabolites found in mobe leaves, is reported to affect the expression of transforming growth factor-beta (TGF-β) protein, thereby increasing fibroblast cell proliferation and accelerating the wound healing process.

AIM: This study aims to determine the wound healing activity of nanoemulgel-containing ethanol extract of mobe leaves.

MATERIALS AND METHODS: The method used in this study was experimental using chemicals ethanol 96%, Carbopol 940, PEG 400, Propylene glycol, Methyl Paraben, Propyl Paraben, Triethanolamine, Aqua Destillata. Mode leaves which were taken purposively were then formulated in nanoemulgel preparations which were tested for wound healing in male rats. The nanoemulgel preparation was then evaluated which included homogeneity, emulsion type, pH, viscosity, dispersion, and measurement of the nanoemulgel globules of mobe leaf extract, stability of the nanoemulgel preparation. Tests for the healing effect of burns were carried out on male rats for 14 days.

RESULTS: Mobe leaves ethanol extract can be formulated into nanoemulgel dosage forms. This study showed wound healing activity of nanoemulgels with concentration variation of mobe leaves ethanol extract. The percentage of wound diameter reducing and fibroblast cells value were showed to increase and significantly different to negative control ($p < 0.05$) in 14 days. Platelet-derived growth factor (PDGF)-BB and TGF-β1 immunoexpression evaluation result showed significantly different to Blanko group ($p < 0.05$) in 14-day observation.

CONCLUSION: From this study, nanoemulgel mobe can stimulate more fibroblast cell proliferation by greatly expressing TGF-β1 and PDGF BB in burn wounds.

Introduction

Burns are kind of accidents that often occur in everyday life. Burns are a form of tissue damage caused by contact between the skin and heat sources such as fire, hot water, chemicals, electricity, and radiation [1]. Burns occur on the skin, mucous membranes, respiratory tract, and gastrointestinal tract. Symptoms include pain, swelling, red, blisters due to increased permeability of blood vessels [2]. It can be treated by minimizing tissue damage, providing tissue perfusion, adequate oxygenation, and nutrition, as well as a moist wound healing environment to restore anatomical continuity and characteristics of the affected part [3]. One of the current projects is looking at natural compounds that might help in wound healing [4]. Wound care with natural components is becoming more popular these days due to the fact that it has less side effects than conventional therapies and the time it takes for wound healing with natural materials is getting faster [5].

One of the natural ingredients that have the potential to be developed as a wound medicine is mobe leaves (*Artocarpus lakoocha* Roxb) [6]. Flavonoids, tannins, saponins, and glycosides are all found in mobe plants. Flavonoids such as artonin a, artonin b, and artokarpin, which can be inhibitory chemical mediators, are the major component of mobe leaves [7], [8]. Artocarpin has been shown to cause the inflammatory phase to begin sooner [9]. Transforming growth factor-beta (TGF-β) production is increased, fibroblast proliferation and migration is increased, and collagen deposition is increased, resulting in a faster wound healing process [10]. Antiseptics such as flavonoids and tannins play a function. It is vital for preventing bacterial development in wounds during the inflammatory phase, and it can speed up wound healing, capillary blood vessel development, and fibroblast cell creation [11], [12]. Based on this, the development of mobe leaves as a wound medicine is very promising. Another important thing is the dosage form used so that the effectiveness of mobe leaves as a wound medicine becomes increased [13].

Semi solid preparation, including ointments, creams, and gels, offers possibilities for all the better ones, such as extended medication contact and wound protection from extreme diagnostics [14], [15]. The gel's dose form is more convenient to apply, and it spreads more quickly on the skin. The gel is also cooling and hydrating, and it readily enters the skin to produce a healing effect. Gel preparations can help to keep the skin hydrated and prevent dehydration [16]. For now, of course, the nano shape has better efficacy. The smaller the particle size, the easier it will be to penetrate the skin membrane barrier and the better the effect [17]. Therefore, the development of nanoemulgel preparations containing mobe leaf extract must be very interesting to do. However, it should be a concern that the carrier should be easy to apply to the skin, not irritating, and safe to use on the skin [18].

Materials and Methods

Materials

Mobe leaves, ethanol 96% (v/v), carbopol 940, isopropyl myristate, isopropyl alcohol, methylparaben, propyl paraben, triethanolamine, aqua destillata, and bioplacenton® gel.

Animal

The experimental study used 60 rats (*Rattus norvegicus*) in good health and weighing between 150 and 200 g. Rats are housed in plastic cages with a humidity level of 40%–60% and a 12-h dark/light cycle. In addition, rats were fed with pellets produced by Cratachem and water ad libitum. The Universitas Sumatera Utara had granted ethics clearance for this project.

Plant

Sampling was carried out purposively without comparing with the same plants from other areas. Mobe leaves were obtained from Deli Tua, Deli Serdang, North Sumatra, Indonesia. Plant identification was carried out at the Indonesian Institute of Sciences, Biology Research Center, LIPI Bogor.

Ethanol extract preparation

The extract was made by maceration method using 96% ethanol. As much as 1.000 grams of mobe leaves powder is put into a glass container then add 7.5 L of 96% ethanol, cover and leave for 5 days protected from light while occasionally stirring, strain, squeeze, wash the dregs with a liquid filter as much as 2.5 L. Transfer to a closed vessel, leave in a cool place, protected from light for 2 days and then pour or filter. The obtained maserati was then concentrated with a rotary evaporator at a temperature of ± 40ºC until an almost crude extract was obtained [19].

Modification nanoemulgel formulation

Nanoemulgel preparation of ethanol extract from mobe leaves begins with the manufacture of nanoemulsion ethanol extract from mobe leaves first. Then added the nanoemulsion to the gel base to add viscosity and comfort when applied. The following Table 1 shows the preparation of nanoemulgel. Nanoemulgel was preparated using spontaneous emulsification techniques. Tween 80 was mixed with some of the distilled water, then stirred until a thick mass is formed and pale yellow. The water phase was made by mixing an extract solution with a solution of tween 80, methyl parabens, propyl parabens in distilled water, while the oil phase was prepared by dissolving isopropyl myristate and isopropyl alcohol. The oil phase was added to the water phase, then sonicated for 30 min until a clear preparation is formed and then homogenized using a magnetic stirrer at a speed of 2000 rpm for ± 1 h so that nanoemulsion was obtained [20].

Nanoemulgel was obtained by preparing in advance the base of the gel by developing carbopol in distilled water for 24 h, then homogenized with a magnetic stirrer and then adding a few drops of triethanolamine to the carbopol, then the carbopol was homogenized again until a thick and transparent gel was formed. The preparation of nanoemulgel was carried out by mixing the nanoemulsion with the gel portion and then homogenized with a magnetic stirrer at a speed of 2000 rpm for ±10 h until a clear and dark green nanoemulgel was formed [21].

Nanoemulgel evaluation

Nanoemulgel evaluation includes organoleptic, homogeneity test, pH test, particle size analysis, viscosity test, and spreadability.

Organoleptic test

Each formula was stored for 12 weeks at room temperature and observed weekly. The observed are changes in color, smell, shape, and damage or separation of phases [22].

Homogeity test

Visual examination was used to check for homogeneity in all created nanoemulgels. It was

Table 1: Composition of nanoemulgel containing ethanol extract of mobe leaves

Formula	Blanko	F1	F2	F3	F4
Composition (g)					
Ethanol extract of mobe leaves	0	1	3	5	7
Isopropyl myristate	7.5	7.5	7.5	7.5	7.5
Isopropyl alcohol	11.25	11.25	11.25	11.25	11.25
Tween 80	33.75	33.75	33.75	33.75	33.75
Methyl paraben	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02
Aqua destillata	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100
Composition of gel base (g)					
Carbopol 940	1	1	1	1	1
TEA	0.4 or q.s until pH 7	0.4 or q.s until pH 7	0.4 or q.s until pH 7	0.4 or q.s until pH 7	0.4 or q.s until pH 7
Aqua destillata	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

TEA: Triethanolamine

examined for its appearance and the presence of aggregates [22].

pH test

Determination of the pH value of the preparation was carried out using a pH meter which was first calibrated using a neutral standard buffer solution (pH 7.01) and an acidic pH buffer solution (pH 4.01) until the instrument showed the pH value. The sample is made in a concentration of 1% and the electrode is immersed in the solution. The apparatus is left to show a constant pH value [23].

Particle size analysis

The particle size was measured at room temperature using the Particle Size Analyzer at the Universitas Sumatera Utara, Physics Integrated Laboratory. Particles from each nanoemulsion gel formula were determined three times: at the start of the preparation, at the 6th week, and at the 12th week [23].

Viscosity test

Spindle 4 was mounted after 100 cc of nanoemulgel is placed in a tube-shaped container. In test preparations, spindles should be immersed. The viscometer was switched on, and the installed rotors may spin at 60 rpm [23].

Spreadability test

The gel was weighed up to 0.5 g before being placed in the center of a scaled round glass. Other round glass materials are placed on top of the gel, along with ballast, such that the weight of the glass is round and the ballast is 100 g, silent for 1 min, and the diameter of the spread is measured [24].

Burn wound healing test

Rats hair was shaved and cleaned on the area to be injured. Wound making was done after the annexed rats first used 0.1 mL lidocaine 2% as subcutaneously. Burns were made using a metal plate with a diameter of 2 cm which was previously dipped in boiling water (100°C) to be sterilized and cooled for a while then the metal plate was dipped again in boiling water (100°C) and then affixed to the back of the rat for 15 s. A total of 60 male rats wet divided into 6 groups, each group consisting of 5 heads [25]. The rat group was given an ethanol extract nanoemulgel of mobe leaves.

Group I : Negative control was given gel base
Group II : Rats were given F1
Group III : Rats were given F2
Group IV : Rats were given F3
Group V : Rats were given F4
Group VI : Positive control was given bioplacenton®

The nanoemulgel was apllied 2 times a day (wound was not closed). The observation of wound observations was carried out on 1, 3, 7, and 14 days visually by measuring the diameter of the wound until the wound is considered healed (the wound is dry and the diameter of wound is reduced) [26].

Visual observation of wound healing

The diameter of the wound is used as a visual parameter of the healing process. The reduction of wound diameter was calculated by the formula [27]:

$$dx = \frac{dx1 + dx2 + dx3 + dx4}{4}$$

Information:
dx = wound diameter in x-day
dx1 = diameter 1
dx2 = diameter 2
dx3 = diameter 3
dx4 = diameter 4.

Nanoemulgel effect on total fibroblast

The effect of nanoemulgels on the number of fibroblast cells of the test group was determined on days 3, 7, and 14. Tissue samples were obtained from each representative of the test group sacrificed using chloroform on days 3, 7, and 14. The sample was soaked in paraffin medium and stained with hematoxylin eosin. It is then observed under a microscope with a magnification of 400 to see fibrolast cells. The number

of fibroblast cells of each test group is calculated in a predetermined manner [28], [29].

Nanoemulgel effect on PDGF BB and TGF-β1 expression

The proteins expression was obtained using immunohistochemical method. The platelet-derived growth factor (PDGF) BB and TGF-β1 expression were observed and calculated in group test on days 3, 7, and 14. A light binocular microscope was used to count the cells, and the results were given as immunohistoscore [30].

Data analysis

The Statistical Package for the Social Science (SPSS) program 21 was used to analysis of the data. Data are expressed as mean ± SEM. Comparison for more than 2 groups by using one-way ANOVA followed by *post hoc* Tukey's test. Statistical significance was set at $p < 0.05$ [31].

Results

Nanoemulgel evaluation

Nanoemulgel has been successfully created based on modified formulas [Table 1]. To see the quality of nanoemulgel, evaluation was carried out, namely organoleptic test, homogeneity test, pH test, particle size analysis, viscosity test, and spreadability. The test results of the nanoemulgel will be explained in this section.

Examination of nanoemulgel organoleptis was done by observing odors, colors, and homogeneity of each formula over 12 weeks of storage [Table 2].

Table 2: Organoleptic evaluation of nanoemulgel

Samples	Parameter	Observation (weeks)			
		0	4	8	12
Blanko	Color	Peach	Peach	Peach	Peach
	Odor	Specific	Specific	Specific	Specific
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F1	Color	Dark green	Dark green	Dark green	Dark green
	Odor	Extract odor	Extract odor	Extract odor	Extract odor
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Color	Dark green	Dark green	Dark green	Dark green
	Odor	Extract odor	Extract odor	Extract odor	Extract odor
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Color	Dark green	Dark green	Dark green	Dark green
	Odor	Extract odor	Extract odor	Extract odor	Extract odor
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F4	Color	Dark green	Dark green	Dark green	Dark green
	Odor	Extract odor	Extract odor	Extract odor	Extract odor
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous

The four nanoemulgel formulas have similarities in smell (typical extract), green color (dark green), and good level of homogeneity. All four formulas give the same result to organoleptic parameters because each formula uses the same composition except the active substance (extract). This means that the addition of extracts to a concentration of 7% has no effect on nanoemulgel organoleptis.

pH testing on nanoemulgels aims to predict the safety of nanoemulgels when applied to the skin [Table 3]. The pH measurement was determined using a pH meter 3 times/formula in weeks 0, 6, and 12.

Table 3: pH value of nanoemulgel

Samples	pH value		
	0	6	12
Blanko	5.06 ± 0.11	6.22 ± 0.13	7.46 ± 0.15
F1	5.13 ± 0.05	6.33 ± 0.05	7.56 ± 0.05
F2	5.23 ± 0.05	6.33 ± 0.05	7.63 ± 0.11
F3	5.32 ± 0.05	6.26 ± 0.12	7.66 ± 0.05
F4	5.41 ± 0.11	6.51 ± 0.05	7.72 ± 0.12

The data displayed is the result of sample measurement (mean ± SD, n = 3). SD: Standard deviation.

Based on the results obtained, there was a change in the degree of acidity of the pH of each nanoemulgel in storage for 12 weeks. The longer the storage time, the pH of the nanoemulgel increases.

Observations of particle size were conducted at the Nanomedicine Laboratory of the USU Faculty of Pharmacy using the FRITSCH Analysette 22 NanoTec Particle Size Analyzer tool at room temperature. Average results measurement of particle size and its changes during 0, 6, and 12 weeks of storage at room temperature [Table 4].

Table 4 : Particle size of nanoemulgel

Samples	Particle size (nm)		
	0	6	12
Blanko	40.32 ± 0.18	219.24 ± 0.72	486.21 ± 0.31
F1	50.44 ± 0.23	590.55 ± 0.41	960.33 ± 0.52
F2	60.51 ± 0.45	234.67 ± 0.71	536.49 ± 0.19
F3	80.78 ± 0.31	569.87 ± 0.32	866.88 ± 0.71
F4	90.12 ± 0.17	339.12 ± 0.38	512.53 ± 0.31

The data displayed is the result of sample measurement (mean ± SD, n = 3). SD: Standard deviation.

The particle size of each formula's nanoemulgel increases with the length of storage time of 12 weeks. For example, nanoemulgel (F4) experienced an increase in particle size ranging from 90.12 ± 0.17 nm (week 0), 339.12 ± 0.38 nm (week 6), and to 512 ± 0.31 nm (week 12). However, the result of this increase in particle size is still within the nanometer size range of each formula.

Measurements of nanoemulgel stability during storage can also be known from changes in viscosity over a period of 12 weeks. Viscosity determination was done using Brookfield NDJ-8S Viscometer tool with a speed of 60 rpm for nanoemulgel on weeks 0, 6, and 12 [Table 4].

According to the findings of the viscosity data, each formula's seeing tends to improve with storage. For example, the viscosity of nanoemulgel (F4) was 974.77 ± 0.73 (week 0), 1007.21 ± 0.61 (week 6), and 1225.92 ± 0.78 (week 12).

Related to viscosity, the spreadability of nanoemulgel must be determined. Spreadability is an important characteristic in formulations that guarantees the ease when nanoemulgel is applied to the skin [Table 5].

Based on the test results, the dispersion obtained tends to be homogeneous, namely in the range

Table 5: Viscosity of nanoemulgel

Samples	Viscosity (mPa/s)		
	0	6	12
Blanko	182.51 ± 0.83	197.53 ± 0.31	217.52 ± 0.77
F1	320.12 ± 0.34	362.56 ± 0.68	385.32 ± 0.92
F2	912.92 ± 0.31	969.67 ± 0.45	1095.19 ± 0.88
F3	869.32 ± 0.65	922.21 ± 0.38	972.50 ± 0.32
F4	974.77 ± 0.73	1007.21 ± 0.61	1225.92 ± 0.78

The data displayed is the result of sample measurement (mean ± SD, n = 3). SD: Standard deviation.

of 5.0–6.2. That is, the addition of the concentration of mobe leaf extract did not significantly affect the quality of the nanoemulgel.

Wound healing activity of nanoemulgel

The wound-healing activity of nanoemulgel was evaluated as *in vivo* in burn-treated rats [Figure 1]. This evaluation lasted for 14 days, taking into account parameters such as wound diameter, increase in fibroblast cells, and the effect of nanoemulgel on the expression of PDGF BB and TGF-β1 proteins [Table 6].

Table 6: Spreadability of nanoemulgel

Samples	Diameter of spreadability (cm)				
	0 g	25 g	50 g	75 g	100 g
Blanko	5.5	5.8	5.9	6.0	6.2
F1	5.4	5.6	5.7	5.8	5.9
F2	5.5	5.6	5.7	5.9	6.0
F3	5.3	5.4	5.5	5.6	5.8
F4	5.5	5.8	5.9	6.0	6.2

According to Table 6, wound healing effect of nanoemulgel contain ethanol extract of mobe leaves was obtained. The percentage of reducing wound diameter value was increased in nanoemulgel group (F1, F2, F3, and F4) if compared to Blanko group, this effect was significantly different ($p < 0.05$). This happened at each observation time (days 3, 7, and 14). However, if the result of nanoemulgel group compared to positive group in observation time (days 3, 7, and 14), this effect was not significantly different ($p > 0.05$).

Fibroblast cell is taken a side to wound healing process [32]. This study was showed the fibrolast cells value after treatment using nanoemulgel groups was increased in observation times compared to Blanko group and significantly different ($p < 0.05$). However, only the F4 group was not significantly different from the positive group ($p > 0.05$). It is mean that the F4 group has the same wound healing activity with positive group.

This was explained by the activity of the nanoemulgel group increasing the expression of PDGF BB and TGF-β1 proteins (Table 7). The proteins expression was increased during observation time. The nanoemulgel groups have activity to increase expression of PDGF BB and TGF-β1 compared to Blanko group; this is significantly different ($p < 0.05$). However, only F1 group was significantly different compared to positive group ($p < 0.05$) to increase TGF- β1. This explains that the extract content in nanoemulgel affects the activity of increasing protein expression.

Discussion

Tissue injury induced by contact with a heat source such as fire, electricity, or dangerous chemicals is known as burns [33]. Burns damage the skin as well as other tissues including blood vessels, nerves, tendons, and bones, increasing the risk of infection. Second-degree burns have damage to the epidermis, although there are still many skin organs above the corium/dermis, such as hair follicles and sebaceous glands, according to histology [34]. Natural compounds that are anti-inflammatory, antioxidant, and antibacterial can be used to treat burns [35].

Mobe leaves are a natural component that has been reported to have the ability to speed up the healing of wounds [36]. Mobe leaves were processed into nanoemulgel preparations with varied concentrations of 1%, 3%, 5%, and 7% in this investigation. Nanoemulgel provides benefits such as good skin adherence and high solubility, allowing the medication concentration to permeate the skin more deeply [37], [38]. Nanoemulgel also helps manage drug release by extending the action of the medicine, which is beneficial for pharmaceuticals and active compounds with poor solubility [39].

Based on the research results, nanoemulgel containing ethanolic extract of mobe leaves was successfully made. The nanoemulgels were evaluated to determine their suitability. Several parameters tested were organoleptic, homogeneity, pH test, particle size, viscosity, and spreadability test. During the 12-week storage period, all assessments were completed. The examination took place three times, at weeks 0, 6, and 12. The formulations generated have no physical or chemical difficulties based on the data obtained. The thing to note is the increase in the particle size of each formula [Table 3]. Particle size is important because it relates to drug penetration into the skin. The smaller the particle size will increase the surface area contact, the higher the contact surface area, the faster the drug substance enters and is absorbed into the skin so that it can produce a optimally desired [22].

Nanoemulgel containing ethanol extract of mobe leaves was applied to rats that had been previously injured (burns). To see the ability of the extract on nanoemulgel, the burn healing process was observed for 14 days through several parameters such as a decrease in wound diameter, the number of fibroblast cells, and the expression of PDGF BB and TGF- β1 proteins. As the number of fibroblast cells rises, the wound's width will shrink [40]. Fibroblasts are cells that play a vital part in wound healing, such as the breakdown of a fibrin clot, the formation of a new extracellular matrix, and the formation of collagen structures to support and closure wounds [41], [42]. According to Table 6, it can be seen the fibrolast cells value after treatment using nanoemulgel groups was increased in observation times compared to Blanko

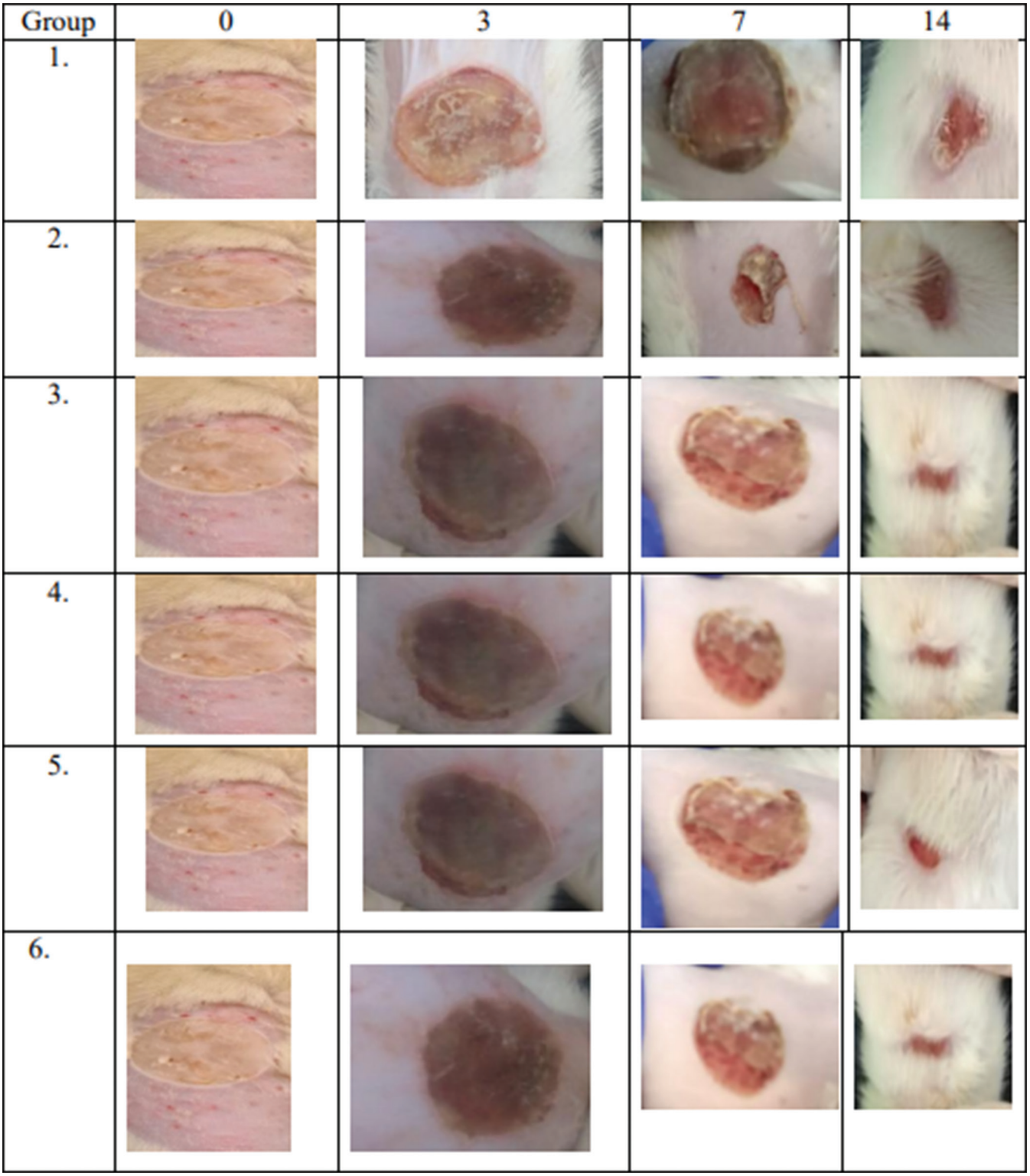

Figure 1: Burn diameter reduction

group and significantly different ($p < 0.05$). However, only the F4 group was not significantly different from the positive group ($p > 0.05$). It means that the F4 group has the same wound healing activity with positive group.

There are various factors influencing fibroblast proliferation. Observations under a microscope said to express TGF if the results of the IHS gave dark brown, medium brown, and purplish light brown while those that did not express purple. The two most important factors are TGF-β1 protein and PDGF BB [43]. PDGF and TGF- β are secreted by platelets as soon as they are injured [44]. PDGF stimulates fibroblast proliferation, which leads to the formation of extracellular matrix. PDGF BB has been identified as a significant mediator in wound healing and tissue repair as a proliferative factor [45]. TGF-β, like PDGF, plays a vital function. TGF-β stimulates fibroblasts and cell chemotaxis, modulates collagen and collagenase expression, and

Table 7: Nanoemulgel effect toward wound diameter, fibroblast cells value, and proteins expression

Parameter	Sample					
	Blanko	F1	F2	F3	F4	Positive group
RV (%)						
Day-3	2.33 ± 0.15	3.64 ± 0.11	3.94 ± 0.32	3.94 ± 0.32	3.86 ± 0.36	3.16 ± 0.36
Day-7	32.82 ± 2.91	44.03 ± 1.58	42.81 ± 1.92	42.81 ± 1.92	45.53 ± 2.64	36.23 ± 2.38
Day-14	45.2 ± 3.23	65.6 ± 1.51	66.43 ± 2.23	75.24 ± 2.16	76.86 ± 1.92	64.61 ± 2.07
FV (cell/field)						
Day-3	19.34 ± 4.84	23.66 ± 2.30	32.46 ± 3.28	34.67 ± 2.54	31.24 ± 6.30	24.41 ± 2.30
Day-7	46.62 ± 5.85	57.23 ± 3.27	57.44 ± 2.96	59.81 ± 5.43	62.83 ± 6.54	63.23 ± 5.89
Day-14	66.31 ± 12.41	90.61 ± 9.15	92.88 ± 3.46	94.25 ± 3.86	97.41 ± 4.81	88.86 ± 3.96
PDGF BB (cell/field)						
Day-3	19.48 ± 5.54	22.43 ± 1.56	34.22 ± 1.43	34.22 ± 2.59	31.26 ± 6.30	25.22 ± 2.91
Day-7	42.41 ± 2.52	57.25 ± 3.21	57.43 ± 2.96	59.86 ± 5.03	64.64 ± 3.74	63.61 ± 3.94
Day-14	62.62 ± 3.31	90.67 ± 9.15	94.64 ± 3.36	95.62 ± 2.02	97.43 ± 6.30	90.62 ± 6.26
TGF β1 (cell/field)						
Day-3	20.05 ± 2.54	23.61 ± 1.51	31.84 ± 3.21	25.65 ± 1.99	22.62 ± 1.31	25.32 ± 2.15
Day-7	43.44 ± 1.58	57.21 ± 3.39	65.45 ± 8.64	66.62 ± 1.56	64.62 ± 3.94	63.23 ± 4.69
Day-14	63.53 ± 2.54	87.83 ± 4.63	96.81 ± 2.94	99.21 ± 6.83	100.81 ± 6.83	90.61 ± 6.46

The data displayed is the result of sample measurement (mean ± SD, n = 3). F1: Nanoemulgel with 1% extract ethanol of mobe leaves, F2: Nanoemulgel with 3% extract ethanol of mobe leaves, F3: Nanoemulgel with 5% extract ethanol of mobe leaves, F4: Nanoemulgel with 7% extract ethanol of mobe leaves. TGF β1: Transforming growth factor-beta 1, PDGF BB: Platelet-derived growth factor-BB, RV: Reducing value of wound diameter, FV: Fibroblast cells value.

creates matrix-producing cells for deposition, resulting in fast release of new connective tissue at the wound site during the rapid proliferative phase followed by an inflammatory phase [46], [47], [48]. The nanoemulgel groups have activity to increase expression of PDGF BB and TGF-β1 compared to Blanko group; this is significantly different ($p < 0.05$). However, only F1 group was significantly different compared to positive group ($p < 0.05$) to increase TGF- β1 [Table 6]. This explains that the extract content in nanoemulgel affects the activity of increasing proteins expression.

Secondary metabolites found in mobe leaves include flavonoids, tannins, saponins, and glycosides [49]. Flavonoids and tannins are antiseptics that protect wounds from bacterial development during the inflammatory phase and can enhance the number of blood vessel and fibroblast cell creation, causing wound healing to proceed swiftly [50]. Flavonoids also have a role in the proliferative phase and tissue remodeling, notably by enhancing vascularization to ensure that the wounded tissue and cells receive the greatest amount of oxygen and nutrients [51]. Collagen production rises as a result, speeding up the wound healing process [52]. Furthermore, it has been observed that artocarpine can activate the inflammatory phase early by enhancing TGF-β production, fibroblast proliferation and migration, and collagen deposition, resulting in faster wound healing processes [53], [54]. This supports the activity of mobe leaves in accelerating the wound healing process.

Conclusion

All preparation formulas of nanoemulgel containing mobe leaves ethanol extract meet the quality requirements of nanoemulgel evaluation. The dosage form of nanoemulgel can heal wounds in rats with increase fibroblast cells and increase PDGF BB and TGF-β1 expressions. *Post hoc* Tukey's HSD test results obtained a concentration result of 7% not significantly different from Bioplacenton® as a positive control. The optimum dose of ethanol extract mobe leaves that can cure burn wounds in rats in the dosage form of the nanoemulgel is 7%.

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References

1. Rowan MP, Cancio LC, Elster EC, Burmeister DM, Rose LF, Natesan S, *et al.* Burn wound healing and treatment: Review and advancements. Crit Care. 2015;19(1):1-12. https://doi.org/10.1186/s13054-015-0961-2
   PMid:26067660
2. Wang Y, Beekman J, Hew J, Jackson S, Issler-Fisher AC, Parungao R *et al.* Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring. Adv Drug Deliv Rev. 2018;123:3-17. https://doi.org/10.1016/j.addr.2017.09.018
3. Oryan A, Alemzadeh E, Moshiri A. Burn wound healing: Present concepts, treatment strategies and future directions. J Wound Care. 2017;26(1):5-19. https://doi.org/10.12968/jowc.2017.26.1.5
4. Fahimi S, Abdollahi M, Mortazavi SA, Hajimehdipoor H, Abdolghaffari AH, Rezvanfa MA. Wound healing activity of a traditionally used poly herbal product in a burn wound model in rats. Iran. Red Crescent Med J. 2015;17(9):e19960. https://doi.org/10.5812/ircmj.19960
   PMid:26473072
5. El-Kased RF, Amer RI, Attia D, Elmazar MM. Honey-based hydrogel: *In vitro* and comparative *in vivo* evaluation for burn wound healing. Sci Rep. 2017;7(1):1-11. https://doi.org/10.1038/s41598-017-08771-8
   PMid:28851905
6. Jagtap UB. Bapat VA. Artocarpus: A review of its traditional

uses, phytochemistry and pharmacology. J Ethnopharmacol. 2010;129(2):142-66. https://doi.org/10.1016/j.jep.2010.03.031
PMid:20380874

7. Zaitun Hasibuan PA, Mardiana M. Antioxidant activity of n-hexane, ethyl acetate and ethanol extract from lakoocha leaves (*Artocarpus lacucha* Buch.-Ham) using DPPH Method. Indones J Pharm Clin Res. 2018;1(2):41-7. https://doi.org/10.32734/idjpcr.v1i2.433
8. Gupta AK, Rather MA, Kumar Jha A, Shashank A, Singhal S, Sharma M, *et al. Artocarpus lakoocha* roxb. And artocarpus heterophyllus lam. flowers: New sources of bioactive compounds. Plants. 2020;9(10):1329. https://doi.org/10.3390/plants9101329
9. Lee CW, Ko HH, Lin CC, Chai CY, Chen WT, Yen FL. Artocarpin attenuates ultraviolet B-induced skin damage in hairless mice by antioxidant and anti-inflammatory effect. Food Chem Toxicol. 2013;60:123-9. https://doi.org/10.1016/j.fct.2013.07.029
PMid:23871788
10. Wong SK, Tangah J, Chan HT, Chan EW. Chemistry and pharmacology of artocarpin: An isoprenyl flavone from artocarpus species. Syst Rev Pharm. 2018;9(1):58-63. https://doi.org/10.5530/srp.2018.1.12
11. Bueno FG, Panizzon GP, de Leite Mello EV, Lechtenberg M, Petereit F, de Mello, *et al*. Hydrolyzable tannins from hydroalcoholic extract from poincianella pluviosa stem bark and its wound-healing properties: Phytochemical investigations and influence on in vitro cell physiology of human keratinocytes and dermal fibroblasts. Fitoterapia. 2014;99:252-60. https://doi.org/10.1016/j.fitote.2014.10.007
PMid:25454458
12. Carvalho MT, Araújo-Filho HG, Barreto AS, Quintans-Júnior LC, Quintans JS. Barretoss. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine, 2021;90:153636. https://doi.org/10.1016/j.phymed.2021.153636
PMid:34333340
13. Choudhury H, Gorain B, Pandey M, Chatterjee LA, Sengupta P, Das A, *et al.* Recent update on nanoemulgel as topical drug delivery system. J Pharm Sci. 2017;106(7):1736-51. https://doi.org/10.1016/j.xphs.2017.03.042
PMId:8412398
14. Pachuau L. Recent developments in novel drug delivery systems for wound healing. Expert Opin Drug Deliv. 2015;12(12):1895-909. https://doi.org/10.1517/17425247.2015.1070143
PMid:26289672
15. Liu H, Wang C, Li C, Qin Y, Wang Z, Yang F, *et al.* A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. RSC Adv. 2018;8(14):7533-49. https://doi.org/10.1039/c7ra13510f
16. Morsy MA, Abdel-Latif RG, Nair AB, Venugopala KN, Ahmed AF, Elsewedy HS, *et al.* Preparation and evaluation of atorvastatin-loaded nanoemulgel on wound-healing efficacy. Pharmaceutics. 2019;11(11):1-15. https://doi.org/10.3390/pharmaceutics11110609
PMid:31766305
17. Algahtani MS, Ahmad MZ, Shaikh IA, Abdel-Wahab BA, Nourein IH, Ahmad J. Thymoquinone loaded topical nanoemulgel for wound healing: Formulation design and *in-vivo* evaluation. Molecules. 2021;26(13):1-16. https://doi.org/10.3390/molecules26133863
PMid:34202733
18. Alyoussef A, El-Gogary RI, Ahmed RF, Farid OA, Bakeer RM, Nasr M. The beneficial activity of curcumin and resveratrol loaded in nanoemulgel for healing of burn-induced wounds. J Drug Deliv Sci Technol. 2021;62:102360. https://doi.org/10.1016/j.jddst.2021.102360
19. Rosidah R, Yuandani Y, Widjaja SS, Auliafendri N, Lubis MF, Muhammad M, *et al.* Phytochemicals analysis and immunomodulatory activity of saurauia vulcani korth. Leaves extracts towards raw 264.7 cell. Rasayan J Chem. 2021;14(2):1378-83. https://doi.org/10.31788/rjc.2021.1426075
20. Kathpalia H, Shreya KK. Topical nanoemmigel formulation of boswellia serrata. Indian J Pharm Sci. 2018;80(2):261-7. https://doi.org/:10.4172/pharmaceutical-sciences.1000353
21. Grace XF. Fabrication and characterization of pongamia pinnata leaf and bark extracts loaded nanoemmigel. J Pharm Sci Res. 2021;13(7):369-373.
22. Mulleria SS, Marina K, Ghetia SM. Formulation, optimization and *in vitro* evaluation of apremilast nanoemulgel for topical delivery. Int J Pharm Investig. 2021;11(2):230-7. https://doi.org/10.5530/ijpi.2021.2.41
23. Arianto A, Lie DY, Sumaiyah S, Bangun H. Preparation and evaluation of nanoemulgels containing a combination of grape seed oil and anisotriazine as sunscreen. Open Access Maced. J Med Sci. 2020;8(B):994-9. https://doi.org/10.3889/oamjms.2020.5293
24. Algahtani MS, Ahmad MZ, Nourein IH, Albarqi HA, Alyami HS, Alyami MH, *et al.* Preparation and characterization of curcumin nanoemulgel utilizing ultrasonication technique for wound healing: *In vitro, ex vivo*, and *in vivo* evaluation. Gels. 2021;7(4):1-17. https://doi.org/10.3390/gels7040213
25. Farzadinia P, Jofreh N, Khatamsaz S, Movahed A, Akbarzadeh S, Mohammadi M, *et al.* Anti-inflammatory and wound healing activities of aloe vera, honey and milk ointment on second-degree burns in rats. Int J Low Extrem Wounds. 2016;15(3):241-7. https://doi.org/10.1177/1534734616645031
PMid:27217089
26. Amutha K, Selvakumari U. Wound healing activity of methanolic stem extract of *Musa paradisiaca* Linn. (Banana) in Wistar albino rats. Int Wound J. 2016;13(5):763-7. https://doi.org/10.1111/iwj.12371
PMid:25224162
27. Silalahi J, Surbakti C. Burn wound healing activity of hydrolyzed virgin coconut oil. Int J PharmTech Res. 2015;8(1):67-73.
28. Somboonwong J, Kankaisre M, Tantisira B, Tantisira MH. Wound healing activities of different extracts of *Centella asiatica* in incision and burn wound models: An experimental animal study. BMC Complement Altern Med. 2012;12:103. https://doi.org/10.1186/1472-6882-12-103
PMid:22817824
29. Akinci M, Ergul Z, Kantarcioglu M, Tapan S, Ozler M, Gunal A, *et al.* The effect of relaparotomy timing on wound healing in an animal model. Int J Surg. 2014;12(12):1434-8. https://doi.org/10.1016/j.ijsu.2014.10.013
30. Assar DH, Elhabashi N, Mokhbatly AA, Ragab AE, Elbialy ZI, Rizk SA, *et al.* Wound healing potential of licorice extract in rat model: Antioxidants, histopathological, immunohistochemical and gene expression evidences. Biomed Pharmacother. 2021;143:112151. https://doi.org/10.1016/j.biopha.2021.112151
PMid:34507115
31. Zebua N, Sijabat WG, Wulandari IA, Nofriani I, Zai WA, Arista RA, *et al.* Incision wound healing test of ethanolic extract gel from salaon (*Parsonsia alboflavescens* [dennst.] mabb.) leaves in male rats. Open Access Maced J Med Sci. 2021;9(A):776-81. https://doi.org/10.3889/oamjms.2021.6662
32. Jian-Ping D, Jun C, Yu-Fei B, Bang-Xing H, Shang-Bin G, Li-Li J. Effects of pearl powder extract and its fractions on fibroblast function relevant to wound repair. Pharm Biol. 2010;48(2):122-7. https://doi.org/10.3109/13880200903046211
PMid:20645827
33. Alemzadeh E, Oryan A, Mohammadi AA. Hyaluronic acid hydrogel loaded by adipose stem cells enhances wound healing

by modulating IL-1β, TGF-β1, and bFGF in burn wound model in rat. J Biomed Mater Res B Appl Biomater. 2020;108(2):555-67. https://doi.org/10.1002/jbm.b.34411
PMid:31081996

34. Pavliuk B, Stechyshyn I, Kramar S, Chubka M, Hroshovyi T. Therapeutic efficacy of the developed gel "Xeliogel" on a burn wound model in rats. Pol Merkur Lekarski. 2020;48(287)331-4.
PMid:33130793
35. Momtaz S, Dibaj M, Abdollahi A, Amin G, Bahramsoltani R, Abdollahi M, *et al.* Wound healing activity of the flowers of *Lilium candidum* L. In burn wound model in rats. J Med Plants. 2020;19(73):109-18. https://doi.org/10.29252/jmp.1.73.109
36. Sonkar KS, Pachauri M, Kumar A, Shukla A, Patel M, Jagannadham MV. Heme-peroxidase from medicinal plant *Artocarpus lakoocha*: Purification, characterization and wound healing studies. Biocatal Agric Biotechnol. 2015;4(2):180-90. https://doi.org/10.1016/j.bcab.2015.03.002
37. Ahmad J, Gautam A, Komath S, Bano M, Garg A, Jain K. Topical nano-emulgel for skin disorders: Formulation approach and characterization. Recent Pat Antiinfect Drug Discov. 2018;14(1):36-48. https://doi.org/10.2174/1574891x14666181129115213
38. Elmarzugi NA, Chellapa P, Mohamed AT, Keleb EI, Elmahgoubi A, Eid AM, *et al*. Nanoemulsion and nanoemulgel as a topical formulation. IOSR J Pharm. 2015;5(10):43-7.
39. Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. Int J Pharma. 2017;526(1-2):353-65. https://doi.org/10.1016/j.ijpharm.2017.04.068
PMid:28461261
40. Fronza M, Heinzmann B, Hamburger M, Laufer S, Merfort I. Determination of the wound healing effect of Calendula extracts using the scratch assay with 3T3 fibroblasts. J Ethnopharmacol. 2009;126(3):463-7. https://doi.org/10.1016/j.jep.2009.09.014
PMid:19781615
41. Adetutu A, Morgan WA, Corcoran O. Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extracts of Bridelia ferruginea leaf, a wound-healing plant of Nigeria. J Ethnopharmacol. 2011;133(1):116-9. https://doi.org/10.1016/j.jep.2010.09.011
PMid:20863876
42. Davoudi-Kiakalayeh A, Mohammadi R, Pourfathollah AA, Siery Z, Davoudi-Kiakalayeh S. Alloimmunization in thalassemia patients: New insight for healthcare. Int J Prev Med. 2017;8:101. https://doi.org/10.4103/ijpvm.IJPVM
PMid:29291043
43. Gallego-Muñoz P, Ibares-Frías L, Valsero-Blanco MC, Cantalapiedra-Rodriguez R, Merayo-Lloves J, Martínez-García MC. Effects of TGFβ1, PDGF-BB, and bFGF, on human corneal fibroblasts proliferation and differentiation during stromal repair. Cytokine. 2017;96:94-101. https://doi.org/10.1016/j.cyto.2017.03.011
PMid:28390267
44. Park SA, Raghunathan VK, Shah NM, Teixeira L, Motta MJ, Covert J, *et al.* PDGF-BB does not accelerate healing in diabetic mice with splinted skin wounds. PLoS One. 2014;9(8):e104447. https://doi.org/10.1371/journal.pone.0104447
PMid:25121729
45. Deptuła M, Karpowicz P, Wardowska A, Sass P, Sosnowski P, Mieczkowska A, *et al.* Development of a peptide derived from platelet-derived growth factor (PDGF-BB) into a potential drug candidate for the treatment of wounds. Adv Wound Care. 2020;9(12):657-75. https://doi.org/10.1089/wound.2019.1051
PMid:33124966
46. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen. 2008;16(5):585-601. https://doi.org/10.1111/j.1524-475X.2008.00410.x
PMid:19128254
47. Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-β family in wound healing, burns and scarring: A review. Int. J. Burns Trauma. 2012;2(1):18-28.
PMid:22928164
48. Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-β) isoforms in wound healing and fibrosis. Wound Repair Regen. 2016;24(2):215-22. https://doi.org/10.1111/wrr.12398
PMid:26704519
49. Singhatong S, Leelarungrayub D, Chaiyasut C. Antioxidant and toxicity activities of artocarpus lakoocha roxb. Heartwood extract. J Med Plants Res. 2010;4(10):947-53. https://doi.org/10.5897/JMPR10.133
50. Pang Y, Zhang Y, Huang L, Xu L, Wang K, Wang D, *et al.* Effects and mechanisms of total flavonoids from *Blumea balsamifera* (L.) DC. On skin wound in rats. Int J Mol Sci. 2017;18(12):2766. https://doi.org/10.3390/ijms18122766
PMid:29257119
51. Li W, Kandhare AD, Mukherjee AA, Bodhankar SL. Hesperidin, a plant flavonoid accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats: Role of TGF-B/SMADS and ANG-1/TIE-2 signaling pathways. EXCLI J. 2018;17:399-419. https://doi.org/10.17179/excli2018-1036
PMid:29805347
52. Nazaruk J, Galicka A. The influence of selected flavonoids from the leaves of *Cirsium palustre* (L.) Scop. On collagen expression in human skin fibroblasts. Phyther Res. 2014;28(9):1399-405. https://doi.org/10.1002/ptr.5143
PMid:24643916
53. Pereira-da-Silva G, Roque-Barreira MC, Van Damme EJ. Artin M. A rational substitution for the names artocarpin and KM+. Immunol Lett. 2008;119(1-2):114-5. https://doi.org/10.1016/j.imlet.2008.06.002
PMid:18602950
54. Daud NN, Septama A, Simbak N, Rahmi E. The phytochemical and pharmacological properties of artocarpin from *Artocarpus heterophyllus*. Asian Pac J Trop Med. 2020;13(1):1-7. https://doi.org/10.4103/1995-7645.273567