Original Article

The potential healing effect of *Kleinhovia hospita* L. leaves extract on second-degree burns in rats

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ABSTRACT

One of the issues in medical research is the management of burn wounds. New wound healing chemicals are hailed for their anti-inflammatory, antibacterial, and antioxidant properties, which help to speed up the healing process. *Kleinhovia hospita* L. has been widely used as traditional medicine by the Komering tribe, in South Sumatra, Indonesia. This research aims to screen the anti-inflammatory and antioxidant activity of *K.hospita* L. leaf extracts in vitro and to determine the healing effect of *K.hospita* L. leaf extract on 2nd-degree burns in rats. The screening activity of anti-inflammatory was used in the human red blood cell membrane stabilization (HRBC) assay and the antioxidant capacity was assessed by ferric reducing antioxidant power (FRAP) assay. The 2nd-degree wounds were induced in 5 groups of 6 rats each. Group 1 as the second-degree burn model group, and groups 2-5 received standard drug (lanakeloid® dose 1g/kg BW), and ethanolic leaves extract of *K.hospita* L. with doses 250, 500, and 750mg/kg BW, respectively. Parameters observed included the percentage of burn healing recovery, time total recovery, and skin histopathology. The best result of anti-inflammatory and antioxidant activity is in the ethanolic extract of *K.hospita* L. with 85.46% stabilization and antioxidant capacity with an EC_{50} value is 71.49 ppm. The best wound healing effect is the rats treated with 750 mg/Kg BW ethanolic extract with 100% recovery on the 16th day. Histopathological features on the 14th day show epithelial and collagen formation. Group 5 shows the best epithelialization compared to the lanakeloid® group.

Keywords: *Kleinhovia hospita L.*, HRBC, FRAP, Epithelial, Phytopharmacology

Introduction

One of the major health problems is burned wounds that affect communities worldwide. Excessive inflammation in the burn location causes local tissue edema, neutrophil activation, and increased vascular permeability, all of which induce local tissue damage [1]. The ultimate goal of burn care and therapy is to avoid infection while promoting quick wound-healing, and epithelialization, and also reducing functional and aesthetic complications [2].

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Burn wounds according to the severity of the wound are classified into 1st, 2nd, and 3rd-degree burns [3, 4]. First-degree burns only affect the outer layer of the epidermis, the skin is red, slightly edematous, and painful. Second-degree burns affect the epidermis and part of the dermis, severe pain, forming water-filled bubbles, and edema [5, 6]. Third-degree burns affect all layers of the skin and can reach the underlying tissue covering the entire thickness of the skin (epidermis and dermis) to subcutis, muscle, and bone tissue [7].

New wound healing chemicals are hailed for their anti-inflammatory, antibacterial, and antioxidant properties, which help to speed up the healing process [8, 9]. One of the plants that have the potential as medicinal ingredients is *Kleinhovia hospita* L. *K.hospita* L. has been widely used as a traditional medicine by the Komering tribe, in South Sumatra, Indonesia to treat inflammation due to tumors, ulcers, and polyps. *K.hospita* L. contain alkaloid, flavonoids, saponins, tannins, and steroid [10]. This plant have antibacterial [11-13], antiinflammation [14, 15], and antioxidant [16, 17] activity. In the present study, the

potential healing effect of *K.hospita* L. leaf extract on deep 2nd-degree burn wounds in rats was investigated [18, 19].

Materials and Methods

Material

The leaves of *Kleinhovia hospita* L. were collected from Belitang, South Sumatra, Indonesia. Taxonomic identification of the plant was performed by Andalas University Herbarium (certificate no.332/K-ID/ANDA/VIII/2017).

Extract preparation

The 1 kg of dried leaves were pounded into a powder and then soaked in 96% ethanol (1:10). The maceration process was maintained at room temperature in the amber bottle for 72 hours while being shaken intermittently. The macerate was filtered using Whatman papers and evaporated using a rotary evaporator at 70°C until a thick crude ethanol extract was obtained (CEE). The fractionation process was used in gradual maceration using n-hexane, ethyl acetate, and ethanol to produce n-hexane fraction (HF), ethyl acetate fraction (EAF), and ethanol fraction (EF), respectively. Water extract (WE) is produced with the infusion method. A total of 200 g of Simplicia was boiled with 2 L of distilled water in a ratio of 1:10 at 90 °C for 15 minutes, the water extract was then filtered and then thickened with a freeze dryer. Qualitative identification of phytochemical compounds for Simplicia, CEE, HF, EAF, EF, and WE were used by specific reageants [10]. The CEE, HF, EAF, EF, and WE of K.hospita L. leaves were used for the bioactivity screenings. The best activity of the extract was then used for healing wounds of second-degree burns in rats.

Determination of total flavonoid content

Total flavonoid content was measured spectrophotometrically based on the formation of complex flavonoid-aluminum [20]. The Calibration curve was created using quercetin, rutin, catechin, and kaempferol. The standard was produced in methanol solution at concentrations of 5, 10, 15, 20, and 25 g/mL. CEE, HF, EAF, EF, and WE were produced in a methanol solution at a concentration of 1000 g/mL. One milliliter of $AlCl_3$ 10 % solution and one milliliter of sodium acetate 5 percent were added to one milliliter of the sample solution. The absorbance value was measured after incubating the sample solution for 30 minutes. The total flavonoid content was reported in mg/g of extract as standard equivalents.

Bioactivity screening

Screening antioxidant capacity with FRAP

The antioxidant capacity of the CEE, HF, EAF, EF, and WE of *K.hospita* L. leaves was measured spectrophotometrically [21]. FRAP assay is based on the rapid reduction in ferric-tripyridyltriazine (Fe^{3+}-TPTZ) by antioxidant present in the sample forming ferrous-tripyridyltriazine (Fe^{2+}-TPTZ), a blue-colored product. Standard solutions of $FeSO_4.7H_2O$ ranging in concentration from 100-1000 µmol/L were made. A Solution to make up the FRAP reagent was prepared by mixing 25 mL acetate buffer, 2,5 mL TPTZ solution, and 2,5 mL $FeCl_3.6H_2O$ solution at 37°C [22, 23]. Freshly prepared working FRAP reagent was pipetted 3 ml and mixed with 0.1 ml of the sample extracts (concentration 25-150 µg/L) and mixed thoroughly. Quercetin was used as the antioxidant standard (concentration 2-12 µg/L). Absorbance (A) was measured directly at 599 nm at pH 3.6. All the determinations were performed in triplicates. The reducing power was calculated by Eq. 1 and the half maximum efficient concentration (EC_{50}) value was calculated from the regression of the concentration-reducing power relationship.

$$\text{Reducing power (\%)} = (\text{Abs. sample} / \text{Abs. FeSO4.7H2O}) \times 100\% \quad (1)$$

Screening Anti-inflammatory activity with membrane stabilization assay

Preparation of blood samples

The in vitro anti-inflammatory effect has used the human red blood cell (HRBC) membrane stabilization assay. A volunteer is a healthy man who had not used NSAIDs for two weeks before the trial provided the blood samples. Ten milliliters of blood were centrifuged at 3000 rpm for 10 mins at 27°C. A sterile pipette was used to separate the supernatant. The residual blood cell deposits were cleaned with iso-saline solution (0.85 g of NaCl was dissolved in phosphate buffer pH 7.4 (0.15 M) until the volume of the solution reached 100 mL, then sterilized at 121°C for 2 hours using autoclave) before being centrifuged again. The procedure was carried out four times till the iso-saline was clear. The volume of blood cells was quantified and resuspended in iso-saline to remove remaining residues, yielding a red blood cell suspension with a concentration of 10% v/v [24]. All the determinations were performed in triplicates.

Hypotonicity-induced hemolysis

The 100 and 1000 g/mL of CEE, HF, EAF, EF, and WE of *K.hospita* L. leaves were produced using distilled water, and 2 mL of hyposaline, 1 mL of phosphate buffer, and 0.5 mL of HRBC suspension were added to each concentration. It was incubated at 37°C for 30 minutes before being centrifuged for 20 minutes at 3000 rpm. At 560 nm, the hemoglobin concentration in the supernatant solution was measured spectrophotometrically. The reference standard was sodium diclofenac, and a control blank was created by removing the extracts. The percentage inhibition of hemolysis was calculated according to the modified method described by Parvin *et al*. [25].

$$\text{\% Inhibition of hemolysis} = 100 \times (OD1 - OD2/OD1) \quad (2)$$

Where: OD1 = optical density of hypotonic-buffered saline solution alone. OD2 = optical density of test sample in a hypotonic solution.

Wound healing method

Animals and model preparation

Healthy male Sprague Dawley rats, weighing 200-250 g and aged 8-10 weeks, were used and the seventh day acclimated at the laboratory facility. All animals were maintained with approved animal care operating procedures consistently by the research ethics committee at Ahmad Dahlan University (certificate no.022012033). They were fed the standard pellet rat diet and water ad libitum. They were kept in a polypropylene cage at room temperature in a 12h light-dark cycle.

Rats were anesthetized with 0.02 mL of lidocaine solution (2%) subcutaneously. The skin on the dorsum was shaved off by depilatory cream. The 2nd-degree burn wound was created with a hot iron plate (dimension: 3x2x0.1 cm^3) that was warmed for 5 minutes with boiling water and then placed for 10 seconds on the skin with an equal pressure [26].

Experimental groups and treatments

The twenty-five rats were randomly divided into five groups: second-degree burn model group, standard drug (Lanakeloid®)-treated group (dose 1g/kg BW), and ethanolic leaves extract of *K.hospita* L. with dose 250mg/kg BW, 500mg/kg BW, and 750mg/kg BW. All the animal groups received deep 2nd-degree burns. Lanakeloid® cream was used as the standard drug containing 1% of *Centella asiatica* phytosome. The treatments were applied for 21 days once daily. The control group did not receive any treatment for 21 days. The wound was not covered after the treatment.

Measurement of mean wound area and recovery percentage

The observation of burn wound healing was carried out until the wound healed by measuring the wound area using a caliper at 2-day intervals. Parameters observed in this study include wound surface area, %recovery, epithelialization period, and skin histopathological features in rats. The change in wound surface area on a given day (WSA_{day-x}) was expressed as a percentage of recovery on the first-day wound induction (WAS_{day-0}) using Eq. 3.

$$\text{Recovery}\ (\%) = [(WSA_{day-0} - WSA_{day-x}) / WSA_{day-0})] \times 100 \quad (3)$$

Histological studies

On days 7, 14, and 21 post-wounding, samples of skin tissue were obtained from all groups using a scalpel for histological examination. The tissues of the skin were fixed with 10% formalin. After fixation, the samples were embedded in paraffin, cut using a cryostat microtome into 3 mm frozen sections, and stained with a hematoxylin-eosin reagent. It determined the diameter of epithelial tissue. Under a microscope, the collagen fiber, inflammatory cells, blood vessels, and granulation tissue of the skin were investigated.

Statistical analysis

The data are expressed as mean ± SD. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) with SPSS ver.26 software followed by the Duncan test to detect the intergroup differences. $P < 0.05$ was considered statistically significant. The Pearson's correlation was used to analyze the correlation between antioxidant and anti-inflammatory effects with total flavonoid content.

Results and Discussion

Phytochemical constituents

The identification of phytochemical content in *K.hospita* L. leaf was determined qualitatively using a specific reagent. The screening was used to determine alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids. The data reveal the presence of various constituents in each sample.

The *K.hospita* L. leaf does not contain triterpenoid. Simplicia, crude ethanolic extract, and ethyl acetate fraction have the same phytochemical contents, there are alkaloids, flavonoids, tannins, saponins, and steroids. Ethanol fraction and water extract contain flavonoids, tannins, saponins, and steroids. N-hexane fraction only contains alkaloids and steroid compounds.

The measurement of the total flavonoid content of CEE, HF, EAF, EF, and WE used a colorimetric method with a spectrophotometer. The total flavonoid content of each sample is shown in **Table 1**.

Table 1. Flavonoid content of *K.hospita* L. leaf

Sample	Total flavonoid (mg/g)			
	Quercetin	Rutin	Catechin	Kaempferol
CEE	41.131±0.689[a]	62.667±0.085[a]	53.308±2.175[a]	97.091±0.535[a]
HF	0[e]	0[e]	7.692±0.769[e]	0[e]
EAF	30.096±1.379[b]	59.273±0.535[c]	36.923±2.512[c]	92.182±0.297[b]
EF	17.223±1.053[d]	60.909±0.393[b]	43.076±2.512[b]	74.727±0.296[d]

WE	27.568±2.217[c]	49.818±0.257[d]	24.615±2.512[d]	85.272±0.445[c]

Note: numbers in the same column followed by the different superscript letters ([a,b,c]) are significantly different ($p < 0.05$)

Table 1 shows the total flavonoid content of *K.hospita* L. leaf. Crude ethanolic extract possessed the highest total flavonoid content. Total flavonoids such as quercetin and kaempferol equivalent are presented in the following descending order: CEE>EAF>WE>EF>HF ($p<0.05$). While total flavonoids as rutin and catechin equivalent order were like CEE>EF>EAF>WE>HF ($p<0.05$).

Screening antioxidant capacity

The antioxidant activity of CEE, HF, EAF, and WE of *K.hospita* L. leaf was carried out using the FRAP method. The electron transfer process from the antioxidant to the molecule $Fe(TPTZ)_2^{3+}$, which works as an oxidizing agent and will be reduced to $Fe(TPTZ)_2^{2+}$, is the basis of the FRAP test. The blue color produced by $Fe(TPTZ)_2^{2+}$ can be measured using a UV-Vis spectrophotometer at 599 nm**.**

Antioxidant capacity is expressed by the value of reducing power. The concentration of each sample and reducing power have a linear relationship, the highest concentration of samples has the highest reducing power. CEE has the highest reducing power against DPPH radical.

The half maximum efficient concentration (EC_{50}) value of each sample was calculated from the linear regression of concentration vs % reducing power relationship. The data of the EC_{50} value is shown in **Table 2**.

Table 2. Antioxidant capacity of *K.hospital* L.leaf

Sample	Linear Reg. eq.	EC_{50} (ppm)	Category [27]
Quercetin	Y=2.316x+14,16	15.474±1.819[a]	Very Strong
CEE	Y=0.242x+32.700	71.487±2.049[b]	Strong
HF	Y=0.151x+3.983	304.748±1.423[f]	Inactive
EAF	Y=0.127x+31.370	146.692±1.579[c]	Medium
EF	Y=0.121x+13.770	299.504±1.267[e]	Inactive
WE	Y=0.159x+8.595	260.408±2.740[d]	Inactive

Note: numbers in the same column followed by the different superscript letters ([a,b,c]) are significantly different ($p < 0.05$)

Quercetin as an antioxidant standard showed very strong activity. Here also, CEE showed strong activity and EAF showed medium activity. The other samples, HF, CF, and WE, showed inactive antioxidants. The EC_{50} value of quercetin shows significant differences among the extracts ($p<0.05$)

The lowest FRAP EC_{50} was observed for quercetin, while the extracts and fraction of K.hospita L. leaf order were like CEE<EAF<WE<EF<HF ($p<0.05$). It is clear from the data that CEE showed the best-reducing power than the other extract and fractions of *K.hospita* L. leaf.

Screening anti-inflammatory activity

The findings of the human red blood cell membrane stabilization assay to assess in vitro anti-inflammatory action are presented in **Table 3** *K.hospita* L. leaf extracts and fractions exhibited concentration-dependent anti-inflammatory activity, with the protection percentage increasing with increasing sample concentration.

Table 3. *In vitro* anti-inflammatory activity of *K.hospital* L. leaf

Sample	Percentage Protection	
	100 ppm	1000 ppm
CEE	52.393±0.265[b]	85.460±0.403[b]
HF	17.486±0.174[f]	44.323±0.407[f]
EAF	29.876±0.407[d]	68.353±0.265[d]
EF	33.333±0.556[c]	74.467±0.267[c]
WE	28.456±0.265[e]	65.160±0.697[e]
Sodium diclofenac	73.460±0.187[a]	92.287±0.265[a]

Note: numbers in the same column followed by the different superscript letters ([a,b,c]) are significantly different ($p < 0.05$)

The protection percent of RBC hemolysis from *K.hospita* L. leaf is presented in the following descending order: CEE>EF>EAF>WE>HF ($p<0.05$). At the concentration of 1000 ppm, the CEE produced 85.460±0.403 % inhibition of RBC hemolysis, as compared with 92.287±0.265% produced by the standard drug sodium diclofenac. It is concluded that CEE showed a greater response than other extracts and fractions of *K.hospita* L. leaf.

Correlations

Correlation between antioxidant and anti-inflammatory activity obtained from total flavonoid content. Antioxidant activity of *K.hospita* L. leaf using FRAP assay expressed as EC_{50} value. The lower the EC_{50} value, the greater the antioxidant activity. Correlation between EC50 value and total flavonoid content (as quercetin, rutin, catechin, and kaempferol) were negatively high ($-0.55 \leq r \leq -0.85$, $p < 0.0.1$ for quercetin, catechin, and kaempferol; $p < 0.05$ for rutin), especially between quercetin content based on FRAP EC_{50} value ($r = -0.847$, $p < 0.001$) **(Table 4)**.

Table 4. Pearson's correlation coefficients of antioxidant activities, anti-inflammatory activities, and flavonoid content

Response	Protection_HRBC	FRAP_EC50	Rutin.eq	Catechin.eq	Quercetin.eq	Kaempferol.eq
Protection_HRBC	1	-.679**	.918**	.971**	.865**	.879**
FRAP_EC50	-.679**	1	-.549*	-.685**	-.847**	-.643**
Rutin. eq	.918**	-.549*	1	.885**	.841**	.968**
Catechin. eq	.971**	-.685**	.885**	1	.796**	.818**
Quercetin. eq	.865**	-.847**	.841**	.796**	1	.926**
Kaempferol.eq	.879**	-.643**	.968**	.818**	.926**	1

Note: significantly difference (**) ($p < 0.01$)

Anti-inflammatory activity using HRBC membrane stabilization test expressed as protection percentage. The correlation between protection percentage and total flavonoid content (as quercetin, rutin, catechin, and kaempferol) was positively high ($0.87 \leq r \leq 0.97$, $p < 0.001$), especially between catechin content based on HRBC membrane stabilization test ($r = 0.971$, $p < 0.001$). It is concluded that the main compound that has great potential antioxidant activity is quercetin and the anti-inflammatory activity is catechin.

The 2nd degree burns wound healing activity

Based on the results of antioxidant and anti-inflammatory screening activity, crude ethanolic extract of *K.hospita* L. leaf has the best activity. The antioxidant and anti-inflammatory properties can accelerate the wound healing. The results of burn wound healing of *K.hospita* L. leaf are shown in **Tables 5 and 6** and **Figure 1**.

Table 5. Percentage of recovery of wound size in control and treated groups

Group	Recovery (%)							
	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
Burn model	9.576±1.279	16.404±3.736	22.130±2.837	29.129±3.542^{c}	36.779±6.887^{c}	52.878±10.630^{c}	76.495±6.892^{b}	83.416±6.151^{b}
Lanakeloid®	10.273±0.654	22.499±6.514	26.988±9.264	49.520±16.044^{bc}	66.615±7.693^{ab}	83.060±8.323^{ab}	89.908±5.181^{a}	96.632±3.123^{a}
CEE250	8,513±2.749	14.048±1.269	20.960±1.795	31.993±5.272^{bc}	50.671±21.786^{b}	78.802±9.456^{b}	91.908±9.274^{a}	97.756±2.245^{a}
CEE500	10.325±0.852	20.753±1.596	33.020±1.211	45.034±1.898^{b}	75.982±13.846^{ab}	93.995±5.607^{ab}	96.892±2.918^{a}	98.610±1.485^{a}
CEE750	11.344±0.963	26.033±3.680	36.187±5.203	65.578±11.796^{a}	90.249±9.596^{a}	97.518±2.747^{a}	98.878±1.323^{a}	100^{a}

Note: numbers in the same column followed by the different superscript letters (^{a,b,c}) are significantly different ($p < 0.05$)

Table 5 shows the recovery percentage of burn wounds, CEE750 had the highest in every time observation compared to all treatment groups. Furthermore, it was significantly higher than the burn wounds model control group starting on the 8th day ($p<0.05$). A significant increase in the recovery percentage in the CEE750 group indicates the rapidity of it led to burn wound healing. Animals who received Lanakeloid® and CEE had a shorter healing time than the burn model control group ($p<0.01$).

Figure 1 shows the histology of the control and the treated groups at different days of analysis. Histopathologic observation on the 7th day after injury showed severed inflammation, edema, and mild angiogenesis in the control groups (burn wound model and standard drug-treated), granulation tissue and superficial epithelium formation were seen in CEE-treated groups. The mean diameter of the superficial epithelium layer in CEE-treated groups is thicker than in control groups ($p<0.001$) **(Table 6)**.

Table 6. Epithelial size

Group	Mean Diameter±SD (µm)		
	7th day	14th day	21st day
Burn Model	55.483±13.972^{b}	107.000±11.223^{c}	152.000±53.548^{c}

Lanakeloid®	62.980±9.744[b]	158.313±49.376[bc]	244.140±28.680[bc]
CEE250	219.653±49.431[a]	227.490±65.561[b]	238.213±22.073[b]
CEE500	255.033±51.662[a]	256.497±52.452[b]	271.940±53.372[b]
CEE750	262.423±30.557[a]	402.773±44.394[a]	948.557±25.116[a]

Note: numbers in the same column followed by the different superscript letters ([a,b,c]) are significantly different ($p < 0.05$)

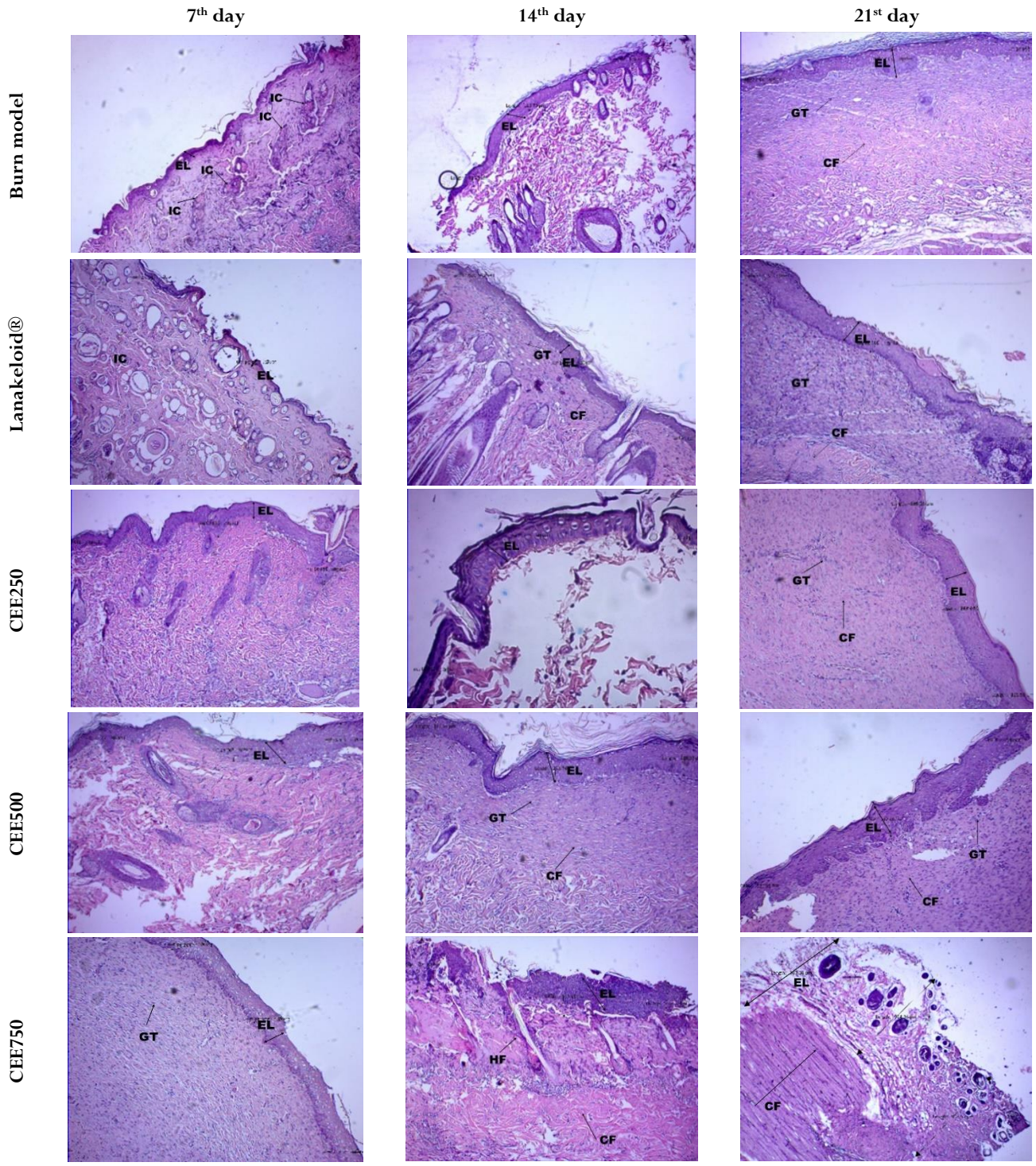

Figure 1. Histopathological observation (skin sections stained with Hematoxylin and Eosin, magnification x 40) of the healing site of skin burn in different groups on the 7th, 14th, and 21st day of the experiment (IC = inflammatory cell; EL = Epithelial layer; GT = Granulation tissue; CF = Collagen fiber; HF = Hair follicle)

Histological sections of granulation tissue from CEE-treated groups revealed better-ordered and thicker epithelial layers as treatment duration increased. On the 14th day, all groups exhibited collagen deposition, fibroblast, and hair follicle. In the control groups, the 7th-day observation revealed more structured epithelial layer bands, more inflammatory cells, and

fewer collagen fibre and fibroblasts. On the 21st day, all groups' wounds exhibited completely developed epithelialization and keratinization. Nearly typical skin appendages were present. There were no visible signs of necrosis or inflammation.

Dermal and epidermal tissues are formed throughout the complicated process of burn healing. It activates several physiological processes at its location, including a rapid inflammatory response initiated shortly after damage, followed by several days of vigorous tissue development [28]. Granulocytes or PMN leucocytes are responsible for this acute inflammation. During this phase, they produce free radicals at the site of inflammation, which can cause tissue damage and inhibit wound healing [29].

Thus, a free radical scavenger can aid in reducing inflammation, hence accelerating tissue creation, re-epithelialization, and epidermis differentiation. Another crucial aspect of the healing process is preventing recurrent deadly wound infections. The antibacterial action can inhibit pathogen development on the skin and promote wound healing. Consequently, the anti-inflammatory, antioxidant, and antibacterial properties of *K.hospita* L. leaf can play a significant role in the healing of burns [30].

K.hospita L. has been widely used as a traditional medicine by the Komering tribe, in South Sumatra, Indonesia to treat inflammation due to tumors, ulcers, and polyps. *K.hospita* L. leaf contains alkaloids, flavonoids, tannins, saponins, and steroids. Crude ethanolic extract of *K.hospita* L. leaf has strong antioxidant activity **(Table 2)**. Flavonoids in *K.hospita* L. leaf play a role major in antioxidant activity [16, 17]. In this study, *K.hospita* L. leaf contains quercetin, rutin, catechin, and kaempferol, especially in crude ethanolic extract (CEE), ethyl acetate fraction (EAF), ethanol fraction, and water extract (WE), while n-hexane fraction (HF) contain only catechin **(Table 1)**. The mechanism of antioxidants in flavonoid compounds is correlated with the number and position of the OH group in the benzene ring. The position of OH groups in the order ortho>para>meta has the highest to the lowest activity of antioxidants [31]. Quercetin, catechin, and rutin have ortho-dihydroxy groups in the structure. But, rutin only has 4 free hydroxy groups, while quercetin and catechin have 5 free hydroxy groups. Besides that, quercetin has ortho-hydroxy-ketone in the C ring. As seen in **Table 4**, quercetin has the highest correlation with EC_{50} of FRAP value ($p<0.001$) and plays a major role in antioxidant activity in *K.hospita* L. leaf.

K.hospita L. leaf also has been reported to have anti-inflammatory [14, 15] and antibacterial activity [12, 13, 32, 33]. In this current study, leaf extracts and fractions of *K.hospita* L. inhibited hypotonicity-induced erythrocyte membrane lysis, demonstrating a membrane-stabilizing activity. Since the erythrocyte membrane is similar to the lysosomal membrane, the stabilization of the erythrocyte membrane suggests that the extracts or fractions may also stabilize lysosomal membranes [34]. Membrane stability prevents serum protein and fluid leakage into the tissue during an inflammatory mediator-induced phase of enhanced permeability [35]. The phytochemical screening test results of this study showed that *K.hospita* L. leaf is abundantly rich in flavonoids, alkaloids, tannins, saponins, and steroids. Anti-inflammatory effects have been observed in flavonoids, tannins, and steroids. It is known that flavonoids, such as quercetin, can reduce acute inflammation. Certain flavonoids have significant inhibitory effects against several enzymes, including tyrosine kinases, protein kinase C, phosphodiesterases, and phospholipase A2 [36]. The anti-inflammatory effect of the extracts/fractions of *K.hospita* L. leaf may be due to the presence of flavonoids, tannins, steroids, alkaloids, and saponins either singly or in combination. This in vitro result suggests that the crude ethanolic extract of *K.hospita* L. leaf possess the highest potential anti-inflammatory activity among other extracts or fractions.

The crude ethanolic extract (CEE) of *K.hospita* L. leaf was observed to effectively accelerate burn wound healing **(Tables 5 and 6)** comparable to the standard drug control group ($p>0.05$). The improved wound healing potential of CEE may be attributable to the free radical-scavenging and anti-inflammatory properties of the phytochemicals included in the extract, and the accelerated rate of wound healing may be due to either the individual or synergistic effect of bioactive compounds. Flavonoids, tannins, steroids, alkaloids, and saponins are the primary components that have a role in speeding wound healing. Flavonoids have anti-inflammatory effects in vitro and in vivo due to their antioxidative impact and modulation of inflammation-related cells. Moreover, their lipid peroxidation decrease can prevent cell damage and necrosis, promote vascularity and circulation, and enhance the viability and strength of collagen. Tannins can accelerate wound healing through a variety of mechanisms, including antioxidant, astringent, antibacterial, and angiogenic activities [29]. Steroids are known to adversely affect wound healing. Steroids reduce inflammation at the stage where oxygen is used by activated inflammatory cells, resulting in tissue hypoxia and lactate production. Steroids also influence the maturation phase of wound healing, notably by inhibiting the synthesis of fibroblasts and collagen [37]. Alkaloids are known to have anti-inflammatory properties through inhibiting cytokine synthesis. Therefore, the accelerated wound-healing ability of alkaloids may be related to the deposition of more fibers with angiogenesis and fewer inflammatory cells in the wound's granulation tissue [38]. It has been observed that saponins have antibacterial, antioxidant, and anti-inflammatory properties. Saponin has been demonstrated to enhance vascular endothelial growth factor and interleukin (IL)-1beta, which is one of the inflammatory cytokines known to cause the recruitment of macrophages at the wound site and accelerate wound healing [39].

Tissue healing involves a synchronized and exquisitely coordinated interaction between several cellular and biochemical components, including homeostasis, inflammation, proliferation, and tissue remodelling [40]. In order to investigate the underlying mechanism of burn wound healing, histological alterations of the healing process were also assessed. The epidermis then proceeded to thicken and ultimately protruded into the dermis **(Figure 1 and Table 6)**. Regulation of collagen expression and wound tensile strength enhancement may

contribute to wound healing benefits. Similarly, an increase in collagen synthesis and angiogenesis has been associated with increased healing activity. On the 7th day, angiogenesis and collagen production were seen in CEE-treated groups **(Figure 1)**. Collagen plays a crucial part in the healing of wound since it is the primary component of connective tissue and provides the structural underpinning for the regenerated tissue. Angiogenesis in granulation tissues enhances blood flow to the wound site, therefore supplying oxygen and nutrients necessary for the healing process, which includes re-epithelialization. Stimulate epithelial cell proliferation is also important for the wound healing process [41]. In CEE-treated groups, the epithelial layer was fully developed by the 7th day, but in the burn model and normal drug-treated groups, the organization begins on the 14th day. Other research revealed that histological examination of treated wounds revealed a high level of fibroblast proliferation, collagen production, and neovascularization, resulting in an increase in wound tensile strength and an accelerated healing process [42].

Conclusion

The crude ethanolic extract of *K.hospita* L. leaf contains abundant flavonoids that have strong antioxidant activity and anti-inflammatory properties. The crude ethanolic extract of *K.hospita* L. leaf can accelerate burn wound healing with significant epithelial and collagen formation.

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