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Original Article

SECOND DEGREE BURN WOUND HEALING ACTIVITY TEST OF ETHANOL EXTRACT MAHOGANY BARK (*SWIETENIA MAHAGONI* (L.) JACQ.)

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ABSTRACT

Objective: This research aims to determine the characteristics and activity of an ethanolic extract of mahogany bark on the healing rate of second-degree burns.

Methods: The animal test consisted of 25 white male rats of the *Sprague Dawley* strain divided into five groups. The positive group was treated by Lanakeloid-E®cream, negative group, and treatment groups with varying dosages (100, 200, and 400 mg/Kg Bodyweight). The parameters observed included the time formed, the scab's shedding time, and the percentage of wound healing. Burn area data were analyzed using the one-way ANOVA test to see if there were differences in the percentage of burns healing between groups.

Results: The results showed that ethanol extract of mahogany bark (*Swietenia mahagoni* (L.) Jacq.) at a dose of 400 mg/Kg Bodyweightwas the best dose for accelerating burn healing with a recovery rate of 85,706% during 14 d. Statistical test results on the percentage of burn injury between negative and positive controls and the test groups had significantly different results ($p<0,05$).

Conclusion: This study concludes that presenting the ethanol extract of mahogany bark (*Swietenia mahagoni* (L.) Jacq) at a dose of 400 mg/Kg Bodyweight shows the best second-degree burns healing.

Keywords: Ethanol extract, Flavonoid, Mahogany bark, Second-degree burns, *Swietenia mahagoni* (L.) Jacq

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INTRODUCTION

A burn is a form of damage or loss of tissue caused by contact with a high-temperature source (such as a flame, hot water, chemicals, electricity, and radiation) [1]. Common causes of burns found in the community, especially in households, are-partial-thickness (second-degree) burns [2]. A sort of burn prevalence in Indonesia is 0.7%. The provinces with the highest prevalence are Papua and Bangka Belitung. Although the burn prevalence is relatively small, it has become a global health issue because of the high mortality and morbidity rates of approximately 1.4% [3].

The principles of treatment in healing the burn are preventing the secondary infection, stimulating the collagen tissue formation, and striving for the remnants of epithelial cells to develop so that they can cover the surface of the wound. The healing process of burns can be classified into three phases: inflammation, proliferation, and maturation [4]. All burns, except superficial (first-degree) burns, need immediate medical treatment because of the risk of infection, dehydration, and other serious complications [5].

Nowadays, herbal or traditional medicine has begun to be widely used by the community. Also, the rising trend "*back to nature*" indicates the community's belief that active compounds from nature are relatively safer than synthetic chemical compounds [6]. Herbal or plant medication is a valuable plant as its taste, aroma, and function can be used for cooking and medication. One of which has the potential as a plant medication to heal the burn is Mahogany bark (MB).

Mahagony is a plant that is classified as a plant medication. Mahogany seeds contain secondary metabolites such as alkaloid, terpenoid, saponin, and tannin, whereas Mahogany bark contains an alkaloid, terpenoid, flavonoid, saponin, and tannin [7]. Flavonoids are useful as they can be antioxidants, antibacterial, and anti-inflammatory [8]. Tannin has antioxidant activity and is useful as a prevention against the wound's infection as tannin has an antiseptic property and its function as a medication for burns by precipitating protein and because tannin has an antibacterial property [9].

Determining the levels of total phenolic, flavonoid, triterpenoid extract of ethanol leaves, bark, and mahogany seeds showed the total phenol compound content in the leaves was 22.06+2.39; the bark was 23.44±2.78 and seed was 1.25±0.14. The content of the total flavonoid compound in the leaves was 21.11±4.85; the bark was 36.87±2.25, and the seeds were 8.94±0.05 [10].

Based on the compound content in the Mahogany (*Swietenia mahagoni* (L.) Jacq.) extract, a scientific approach was conducted to the Mahogany bark (MB) as a burn medication. This information becomes a reason for conducting research using Mahogany bark to accelerate the recovery of the partial-thickness burns (second-degree) on white male rats of the *Sprague-Dawley* strain.

MATERIALS AND METHODS

Plant material

The material used for the study was the mahogany bark (*Swietenia mahagoni* (L.) Jacq.). The samples were obtained from Sriwijaya University Indralaya, Ogan Ilir, South Sumatera. The plant determination of the mahogany was carried out to identify the truth of the plant to be used macroscopically. Determination was conducted at the Botanical Gardens Conservation Center, Bogor, West Java, Indonesia.

Materials

The materials used in this research included: mahogany bark, ethanol 96% (PT. Dira Sonita), aquadest (PT. Dira Sonita, Palembang Indonesia), silica gel TLC plate GF_{254} (Merck®), Lanakeloid-E® (*Centella asiatica* extract and vitamin E), lidocaine (PT. Bernofarm, Jambi Indonesia), male white rat, Veet®, ethanol (PT. Dira Sonita), chloroform (PT. Dira Sonita Palembang Indonesia), butanol (PT. Dira Sonita, Palembang Indonesia), n-hexane (PT. Dira Sonita, Palembang Indonesia), Dragendorff reagent, Mayer reagent, Wagner reagent (PT. Dira Sonita, Palembang Indonesia), and $AlCl_3$ (PT. Dira Sonita, Palembang Indonesia), concentrated H_2SO_4 (Merck and Co), HCl solution 2 N (PT. Bratachem Palembang Indonesia).

Animal testing

This research has received ethical approval with the number 076/kepkrsmhfkunsri/2020. The test animals used were 25 animals

which were divided into 5 groups with 5 animals in each group. The animal testing used in this research were white male rats in *Sprague Dawley* strain that are healthy and have the age 2-3 mo and weigh 100-150 gram.

Extract preparation

The Mahogany bark was obtained from Sriwijaya University, Indralaya, Ogan Ilir, South Sumatera. The fresh bark was cleaned under the running water. The drying process of the material used the sunlight. The simplicia was ground to proceed to the extraction stage. The extraction method used was maceration. 1 kg dried powder of simplicia was macerated in a container protected from the sunlight. The first maceration used 4 L of 96% ethanol for two days and was re-macerated two times with 3 L of 96 % ethanol each for 2x24 h. The macerate was separated using Whatman® No.42 filter paper and evaporated using a rotary evaporator set to 70 °C

Phytochemicals screening of flavonoid

The 500 mg extract was mixed with 5 ml of 70% Ethanol, then stirred and heated for 5 min, and filtered. The obtained filtrate was then added two drops of 2N NaOH. A positive result of flavonoid showed a change in yellow to orange-greenish red, which indicated the contents of flavonoid [8].

Phytochemicals screening of alkaloid, steroid, triterpenoid

The 2 g extract was mixed with 5 ml of 0.05 N ammonia solution in chloroform, which was poured into a test tube, then shaken and filtered. The filtrate was mixed with five drops of 2N sulfuric acid and deposited until two layers formed. The upper layer was used for research. The filtrate was divided into 3 test tubes with a volume of 2.5 ml each. Those tubes were analyzed using Mayer, Wagner, and Dragendorff reagents. The white deposit formed on the Mayer reagent, the brown on Wagner reagent, and the orange on the Dragendorff reagent. Each test resulted in a positive result for alkaloid [11].

Alkaloids and terpenoids were tested by using the lower layer. The lower layer was dripped on a drop plate and let dry. After it was dry, anhydrite acetate acid was added and mixed until homogenous. Then, three drops of concentrated sulfuric acid were added. Green discoloration showed that there was a steroid, yet brownish-red discoloration showed that there was a triterpenoid [12].

Phytochemical's screening of saponin

The 1g of extract was poured into a test tube. Then, 10 ml of hot water was added and heated for 5 min. Let the tube cool, then shake it powerfully. The formation of stable (long-lasting) foam for 10 min at a minimum height of 1 cm showed positive for saponins [12].

Extract activity testing

The animals used in the test were healthy male white rats of the *Sprague-Dawley* strain aged 2-3 mo old, weighed 100–150 g, and were acclimatized toward the laboratory environment for one week. During the acclimatization process, the general condition and weight consideration of rats were observed

Burns making

The tested animals, which had been shaved on the back area using Veet®, were then cleaned by applying 70% alcohol. Before giving the burns induction, the rats were sedated using a subcutaneous injection of 2% lidocaine. The burns were made using an iron plate of 3 x 2 cm size, which had been heated in the boiling water for 5 min, then the rats were inducted on the backs for 10 seconds [13].

Burns healing activity test

The condensed extract was applied to the rats, which had been weighed previously using the analytical balance, which was adjusted according to the dosage of the rats.

Burns healing observation

The initial observation of the burns was conducted for 24 h after burns were made. The following observations were made from days 1-14 with a burn measurement interval of 2 d. The observation parameters included the formation and detachment of scab and % recovery on the tested animals until the burns healed, which could be identified by the wound's closure [14].

The area of burns was measured using the ImageJ®application as one of the quantitative image analysis tools that can be used to measure the surface area.

Data analysis

The test result was analyzed using data processing SPPS software. The mean and standard deviation were calculated from each group in which there were five rats per group. The normality test uses the Shapiro-Wilk statistical testone-way ANOVA test to find whether or not there is a significant difference among experimental groups. If the data are not normally distributed, they can be analyzed using the Mann-Whitney test.

RESULTS AND DISCUSSION

Phytochemical screening

The ethanol extract of the mahogany bark (*Swietenia mahagoni* (L.) Jacq.) was analyzed phytochemically to determine the content of the sample used.

Table 1: Phytochemical screening results of mahogany bark (*Swietenia mahagoni* (L.) Jacq.)

Compound identification	Result
Alkaloid	
Dragendorff	-
Mayer	-
Wagner	-
Flavonoid	+
Steroid	-
Triterpenoid	+
Tanin	+
Saponin	+

(+) contains secondary metabolites; (-) does not contain secondary metabolites

Phytochemical screening was conducted to determine the secondary metabolite in the condensed extract. The results of the phytochemical test of ethanol extract of Mahogany bark can be shown in table 1. Phytochemical analysis of an ethanol extract of mahogany bark revealed the presence of secondary metabolites such as flavonoids, triterpenoid, tannin, and saponin. The identification of flavonoid compounds showed a positive result after adding NaOH, which the discoloration of reddish-orange could identify. It happened as flavonoid forms the reddish-yellow flavylium salts.

The tannin test aimed to determine whether the extract contained the phenol groups, which could be identified from a positive result if its color was blackish green after reacting with $FeCl_3$. $FeCl_3$ can produce that color as it reacts with one of the hydroxyl groups contained in tannin compounds. Following the obtained result, an ethanol extract of Mahogany bark was positive for containing tannin.

The identification of the saponin compound showed a positive result as the constant foam was formed after shaking with hot water.

Saponin has a polar and nonpolar group with an active surface. When shaken, the micelles similar to foam are formed as saponin is dispersed between polar and nonpolar compounds [18].

A qualitative identification using Thin Layer Chromatography was aimed to ensure the presence of compounds that are useful in the burns healing process. Flavonoid compounds were identified using the GF_{254} silica plate with a mobile phase of Butanol: N-Hexane (4:6) and observed under the UV lamps of 254 nm and 366 nm. The observation on the stains using the UV lamps of 254 nm and 366 nm is to observe the interaction between the UV and fluorescence indicator on the silica plate and the interaction between the UV and chromophore groups in the stains [19].

At the UV 254 nm, the plate would be fluorescent in green, and the stain would be dark due to the interaction between the UV light and the fluorescence indicator on the plate. Meanwhile, the stains at the UV 366 nm would be fluorescent in blueish-green, and the plat became dark due to the interaction between the UV light and chromophore groups in the stains. $AlCl_3$ was sprayed to ensure that compounds fluoresced at UV 366 nm were flavonoid compounds [19].

Visible light's yellow identifies flavonoid compounds to brown appearance [8]. The identification of flavonoid compounds at the UV 254 nm was from the appearance of a dark stain. Meanwhile, the fluorescent stain was greenish-yellow at the UV 366 nm after $AlCl_3$ sprayed it. There was a dark stain at the UV 254 nm and a fluorescent stain at the UV 366 nm. It identifies that the flavonoid group can be found in ethanol extract of Mahogany bark.

Extract activity testing

The burn healing activity test of ethanol extract of Mahogany bark was experimentally conducted by using the 25 male white rats Sprague-Dawley line divided into five groups as shown in (table 2). The observed parameters were the time of scab formation and detachment and the % recovery of the burn [14].

Table 2: Provision of test materials

Group	Treatment
Positive	Lanakeloid-E®1 g/kgBW
Negative	Not Given Mahogany Bark Extract
Treatment 1	Dose of 100 mg/KgBW Mahogany Bark Extract
Treatment 2	Dose of 200 mg/KgBW Mahogany Bark Extract
Treatment 3	Dose of 400 mg/KgBW Mahogany Bark Extract

The partial-thickness (second degree) burn was selected in this research as this case was commonly found in the community, especially in the household was the burn with the percentage of the second-degree burn of 73%. This kind of burn requires immediate medical treatment as it can cause infection, dehydration, and other serious implications. The partial-thickness (second degree) burn formation used 3x2 cm size and a 2 mm thick iron plate. The partial-thickness (second degree) burns to cause damage on the epidermis part, a half part of the dermis, and skin appendixes such as hair follicle, sweat glands, which can be seen by skin discoloration in which the skin is paler, blistered and wounded [1].

The rats had been acclimatized before in 7 d so that the rats' condition returned stable and could adapt to the new environment. The rats were shaved on the backs using Veet® cream (potassium thioglycolate, calcium hydroxide, and aloe vera extract). Then they were injected using lidocaine HCl 2% 4 mg/bodyweight subcutaneously to ease the pain during the burn's formation. The selection of lidocaine anesthetic was a strong local anesthetic with rapid action and medium onset around 2 h. Lidocaine is worked by preventing nerve conduction by binding to specific receptors on the Natrium canal, so it causes canal blockage [19].

Lanakeloid® was used as a positive control in this research because the research [20] reported that Lanakeloid® could heal burns within eight days. Lanakeloid® contains 10 mg of Centella Asiatica extract per preparation, and this preparation has been used to accelerate the recovery of the wound as it contains asiaticoside, flavonoid, phenolic, essential oil, saponin, tannin, which can stimulate collagen formation and cell revitalization to accelerate the burns' healing process [21, 22].

The parameters of burn healing in this research were based on the percentage area of burn healing (%recovery) and the detachment of scabs. The healing phase begins with the inflammation process, which is indicated by the swelling as the cause of the migration of inflammatory cells on the wound's area [23]. The next phase is the proliferation phase which generally starts from the 4th day and is indicated by the formation of scabs.

A scab is a dead cell that has dried and mixed with the remaining blood and pus. A scab is functioned to cover the wound and prevent further contamination of the wound's area. On the lower part of the scab, a young layer will force the dead skin above to peel off [22]. The speed at which the formation and detachment of scabs indicate the speed at burn recovery. The faster the time of formation and detachment of the scabs, the better the ethanol extract of Mahogany bark in the burn healing. The 3rd treatment showed scab formation and detachment time was faster than the 1st and 2nd treatments. The time of scabs' formation and detachment can be seen in table 3.

Table 3: Scab observation

Treatment group	Average day	
	Scab formation	Scab release
Positive Group	2	9
Negative Group	4	14
Treatment 1	2	12
Treatment 2	3	12
Treatment 3	2	10

The measurement of the burnt area was observed from day 0 to day 14 in 2 d of time interval by taking the wound's picture using a ruler scale on the side's wound. Then it was calculated quantitatively using the ImageJ® application. The burn healing percentage data were analyzed using the SPSS® application. The reduction result of the burn area can be shown in table 4.

Table 4: The result of observations of burn healing

Treatment group	**mean±SD burn area (cm^2) on day 0**	**mean±SD burn area (cm^2) on day 14**	**Decreased burn area (cm)**	**mean±SD area of burn healing (%)**
Positive Group	5.713±0.049	0.406±0.010	5.307	92.920±0.507
Negative Group	6.122±0.023	2.653±0.079	3.469	56.459±0.806
Treatment 1	6.150±0.051	1.806±0.096	4.344	70.609±0.320
Treatment 2	5.932±0.052	1.157±0.083	4.775	80.420±0.425
Treatment 3	5.783±0.077	0.828±0.076	4.955	85.706±0.461

The data was presented in mean±SD, n=5

Based on the observation data on the formation and detachment of the scabs also the burn healing percentage data (% recovery), the 3rd treatment group was the best treatment group rather than the 1st and 2nd treatment group. As shown in table 4, the % recovery of the 3rd group treatment was 85.706±0.461 %, the time's formation of the scabs on the 2nd day, and the detachment of the scabs on the 10th day. Meanwhile, the % recovery of the 2nd and 1st treatment group's % recovery was 80.420±0.425 % and 70.609±0.320 %.

The ethanol extract of Mahogany bark contains active substances that play the role of the burns healing process, such as flavonoid, tannin, triterpenoid, and saponin. The 3rd treatment group with 40 mg/kg WW dosage had a higher secondary metabolite concentration than the 1st and 2nd treatment, so it was faster healing the burns. The normality test of Shapiro-Wilk on each group showed that p>0.05, which means that the data was normally distributed, and from ANOVA result test to the % recovery of burns showed that p<0,05. It means that the 3rd treatment was significantly different from the 1st and 2nd treatment and negative control, as shown in table 5.

The result ANOVA test on the 3rd treatment group using positive control (Lanakeloid®) showed no significant difference. The 3rd treatment has the same ability in healing the burns as the positive control. The Lanakeloid®, which contains *Centella asiatica* extract, has some secondary metabolites such as alkaloid, alkaloid, flavonoid, tannin, triterpenoid, and saponin can accelerate the healing of the burns. Meanwhile, ethanol extract of Mahogany bark has some secondary metabolites such as flavonoid, tannin, triterpenoid, and saponin (reference).

Table 5: The result of the *one-way anova* statistical test

Group	Significant (0.05)	Meaning
Positive Control vs. Negative Control	0.000	Significantly different
Positive Control vs. Treatment 1	0.001	Significantly different
Positive Control vs. Treatment 2	0.032	Significantly different
Positive Control vs. Treatment 3	0.198	Not Significantly different
Negative Control vs. Treatment 1	0.017	Significantly different
Negative Control vs. Treatment 2	0.000	Significantly different
Negative Control vs. Treatment 3	0.000	Significantly different
Treatment 1 vs. Treatment 2	0.085	Not Significantly different
Treatment 1 vs. Treatment 3	0.011	Significantly different
Treatment 2 vs. Treatment 3	0.341	Not Significantly different

The contents of flavonoid, tannin, and triterpenoid in the ethanol extract of Mahogany bark have antioxidants activity, reducing the free radicals**.** Free radicals can inhibit the proliferation of the cell, the inflammatory reaction, and the contraction of collagen tissues, so they can cause the inhibition of the burns healing process [23]. Flavonoid compound is an active compound that plays a role in the healing process of burns. It can inhibit the growth of bacteria on the living tissues and be useful as an antiseptic and antibacterial by precipitating the protein [24].

Flavonoids can be used as an anti-inflammation by inhibiting cyclooxygenase (COX) and lipoxygenase enzyme, inhibiting leukotriene and prostaglandin synthesis from reducing inflammation and pain. Saponin in the extract of Mahogany bark also can accelerate the migration process of keratinocytes, which plays an important role in the revitalization process and it synthesizes the collage to the wound area.

The negative control group had a significant difference among the 1st, 2nd, and 3rd treatments with a % recovery of 56.459 %. The negative control group was not given the substance that could help accelerate the healing process of burns, so the time of granulation tissue formation was longer than other treatments. The healing process of burns in the negative control group naturally occurs because of the bio-cellular and biochemical process that naturally occurs in the body [33]. Also, there are healthy epithelial cells so that the wound can heal itself in 2-3 w [4].

The initial phase of burns healing is an inflammation phase occurring on 0–5, seen on the 4th day where the *%recovery* is under 20%. The burns cause damage to the tissues' structures and cause bleeding [25]. This inflammation phase is a phase with a higher risk of infection because a liquid appears on the skin, which is caused by the detachment of mediator inflammation on the wound's area. It can be the medium for bacterial growth, so infection and inflammation easily occur.

Neutrophils and monocytes are the first cells migrated to the inflammation area. Various inflammation mediators are such as *prostaglandin*, *interleukin*-1, TNF, TGF-β also the detachment of serotonin and histamine by mast cells and connective tissue, which caused the migration of neutrophils to the wound area. Neutrophils play an important role in phagocytizing the dead tissue, preventing the infection on the wound, and preparing new tissues. Macrophages will also follow the neutrophil and substitute the neutrophil to the wound's area after 48-72 h and stimulate the formation of granulation using fibroblast to form the collagen [22].

After the inflammation process is the proliferation process, fibroblast usually appears on the 3rd day and reaches a peak on the 7th day [26]. Fibroblasts produce the extracellular matrix, which will fill the burns tissues. Macrophages produce growth factors such as PDGF and TGF-β. The proliferation phase ends if the new epithelial cells are formed (re-epithelization), the collagen layer is formed. Also, the scabs are detached. After the scabs are detached, the next process of healing burns is the maturation phase.

Maturation/remodeling is a completing phase of tissues so that the tissues formed are better and stronger. The young collagen formed in the proliferation phase will be changed into stronger collagen with a better structure by collagenase [27]. The presence activities of antioxidant, antibacterial, and anti-inflammation from the ethanol extract of Mahogany bark can accelerate the healing process of the burns.

CONCLUSION

The ethanol extract of Mahoganybark (*Swietenia mahagoni* (L.) Jacq) contains flavonoid, tannin, steroid, and triterpenoid. The variation on the dosages of the ethanol extract of Mahogany Bark (Swietenia mahagoni (L.) Jacq) that affected the burns healing with the dosage of the 400 mg/Kg Body weight showed that the best healing of the second-degree burns using the % recovery of 85.706±0.461 % compared to a dose of 100 mg/Kg Bodyweight and 200 mg/kg Body weight with % recovery 70.609±0.320 % and 80.420±0.425 %.

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AUTHORS CONTRIBUTIONS

All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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