

Effectiveness of Mango Leaf Extract (Mangifera indica L) on Healing of Second Level Cut Wound in Male White Mice (Mus musculus)

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Abstrak

Daun mangga mempunyai beberapa senyawa metabolit sekunder yang memiliki efek farmakologis dan dapat digunakan untuk penyembuhan luka sayat atau luka lainnya. Tujuan penelitian ini adalah untuk mengetahui efektifitas pemberian ekstrak daun mangga terhadap penyembuhan luka sayat pada mencit putih jantan. Penelitian eksperimental ini dilaksanakan di Laboratorium Farmasi Universitas Kader Bangsa dan STIK Siti Khodijah, Palembang pada bulan Juli – Agustus 2021. Daun mangga dimaserasi menggunakan etanol 96 %. Ekstrak yang diperoleh dibuat menjadi salep pada konsentrasi 10, 20, dan 40 %. Salep ekstrak daun mangga, kontrol positif, dan kontrol negatif masing-masing dioleskan dua kali sehari. Panjang luka diukur menggunakan jangka sorong pada hari ke 2, 4, 6, 8, 10, 12, dan 14. Persentase pengurangan panjang luka pada hari ke 14 setelah pemberian salep ekstrak daun mangga 10, 20, dan 40 % masing-masing adalah 14,4±2,07; 17,2±3,70 dan 16,4±4,15 %; sedangkan pada pemberian kontrol positif dan kontrol negatif masing-masing adalah 25,4±3,05 dan 11,4±1,14 %. Terdapat perbedaan yang bermakna antara pemberian salep ekstrak daun mangga pada konsentrasi 20 dan 40 % dibandingkan dengan kontrol negatif ($p<0,05$). Ekstrak daun mangga cukup efektif menyembuhkan luka sayat Level II.

Kata kunci: Bioplacenton, ekstrak daun mangga, luka.

Abstract

Mango leaves have several secondary metabolites which have pharmacological effects and may be used to cure cuts or other injuries. The purpose of this study was to determine the effectiveness of the mango leaf extract on wound healing in male white mice. This experimental research was carried out at the Pharmacy Laboratory, Universitas Kader Bangsa and STIK Siti Khodijah, Palembang in July to August 2021. Mango leaves were macerated using 96% ethanol. The extract obtained was prepared into the ointment at concentrations of 10, 20, and 40%. Mango leaf extract ointment, positive, and negative control were each applied twice daily. Wound length was measured using a caliper on days of 2, 4, 6, 8, 10, 12, and 14. The percentage reduction in wound length on day 14 after administration of mango leaf extract ointment 10, 20, and 40% were 14,4±2.07; 17.2±3.70 and 16.4±4.15%; respectively. While the positive control and negative control were 25.4±3.05 and 11.4±1.14%, respectively. There was a significant difference between the administration of the mango leaf extract ointment at concentrations of 20 and 40% compared to the negative control ($p<0.05$). The mango leaf extract is quite effective in healing Level II cut wounds.

Keywords: *Bioplacenton, mango leaf extract, wound.*

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Introduction

Indonesian people have known various types of medicinal plants and their use to maintain health and treat various diseases since hundred years. Types of plants used as medicine are usually referred to as traditional medicine (Kanon, 2012). One of the plants that are efficacious as medicine is mango leaf plant (*Mangifera indica* L). The leaf of this plant contains secondary metabolites. Utilization of mango is still limited as fruit. The utilization of mango leaves as medicine is still not optimal.

Mango leaves contain compounds such as alkaloids, saponins, tannins, and flavonoids that can heal wounds. The largest content of the mango leaf extract is mangiferin which has been studied by several researchers. It can be used as antioxidant, analgesic, antidiabetic, antitumor, antimicrobial, and increasing stamina

The incidence of injuries in the world throughout the year is increasing, including acute wounds or chronic wounds. In 2009, a study conducted in America stated that the prevalence of injured patients was 350 per 1000 population. The etiology of wounds in patients varies with the data obtained, in term of surgical wounds 113.3 million cases, traumatic wounds 1.6 million cases, abrasions 20.4 million cases, burns 10 million cases, and decubitus ulcers 8.5 million cases (Diligence, 2009). The prevalence of injured patients in Indonesia, according to the Ministry of Health of the Republic of Indonesia in 2013 was 8.2% with the highest number in the province of South Sulawesi, 12.8% and the highest type of wound experienced by the Indonesian population was abrasions at 70.9%. The etiology of the injury was blown away 40.9%, followed by a motorcycle accident 40.6% (Risksdas, 2013).

The mango plant has several medicinal uses. The parts of the mango

plant that have benefits include the roots, skin, and leaves of mangoes that can be used as anti-inflammatory, antioxidant, antidiabetic, antimicrobial and wound healing.

Method

This research was an experimental investigation. Ethical clearance was obtained from the Health Research Ethics Commission, Faculty of Health, Universitas Kader Bangsa Number 310/UKB.FKES/TU.KEPK/2021, dated August 28, 2021. The species of mango was determined by the Herbarium Laboratory of FMIPA Universitas Andalas, Padang.

Equipment used were rotary evaporator, scalpel, caliper, analytical balance, mesh 60 sieve, and glassware commonly used in the laboratory.

The materials used were fresh mango leaves, ethanol, lidocaine, filter paper, alcohol swabs, Vaseline album, distilled water, and Bioplaceton Gel. The experimental animals used were white male mice, *Mus musculus* (Swiss Webster strain).

Preparation of Mango Leaf *Simplicia*. Mango leaves were washed using tap water, dried without being exposed to direct sunlight. This was performed to prevent the growth of mold and to maintain the quality of the sample for a long time. Then, It was chopped into the suitable size. The dried sample was mashed with a blender to obtain a fine powder, then filtered through a 60 mesh sieve and put in a closed vessel.

Preparation of Mango Leaf Extract. The extraction process of mango leaves (*Mangifera indica* L) was carried out by the maceration method using 96% ethanol as solvent. 800 grams of mashed sample was placed into a maceration bottle, then added solvent until the *simplicia* powder was completely immersed. The maceration container was covered with aluminum foil. The mixture

was kept for 3 days and protected from sunlight, and stirred occasionally. After 3 days, the mixture was removed from the bottle and filtered with cotton, evaporated to a constant weight to obtain the mango leaf extract.

Preparation of Ointment. Formulation of the mango leaf extract ointments were as follows. Formula 1, 2, and 3 contain mango leaf extract and vaseline at the ratio of 0.5:4.5, 1:4, and 2:3, respectively.

The mango leaf extract was placed into the mortar and added vaseline step by step, grinded until homogeneous. The ointment was placed in a tightly closed container, and stored at room temperature protected from sunlight.

Alkaloid Test. Two mL of the mango leaf extract solution was added to 5 mL of 2 N HCL. The solution was then divided into 3 test tubes. The first tube was added with 3 drops of Wagner's reagent, the second tube was added with 3 drops of Dragendorff's reagent, and the third tube was added with 3 drops of Mayer's reagent. A positive test is indicated by the formation of an orange to red precipitate using the Wagner reagent, an orange precipitate using the Dragendorff's reagent, a white to yellowish precipitate using the Mayer reagent (Susilowati et al., 2018).

Flavonoid Test. Three drops the extract solution was pipetted and then dropped into a drip plate, then one drop of sulfuric acid (H₂SO₄) added, if the solution changes to yellow, red or brown, the sample was stated as positive for flavonoids.

Saponin Test. One mL of the mango leaf extract solution was added to 10 mL of hot water, then cooled and shaken for 10 seconds. If foam is formed for approximately 10 minutes 1-10 cm in height and does not disappear when 1 drop of 2N HCL is added, it indicates that the tested extract contains saponin.

Steroid and Terpenoid Test. Two mL

of the mango leaf extract solution was added with 10 drops of glacial CH₃COOH, and two drops of concentrated H₂SO₄. The solution was shaken gently and left for a few minutes. Steroids will give a blue or green color while terpenoids will give a red or purple color (Susilowati et al., 2018).

Tannin Test. Two mL of the mango leaf extract solution was added 3-5 drops of 1% iron (III) chloride solution, if there was a dark blue or greenish black color change, it indicated the presence of tannins (Susilowati et al., 2018).

The Treatment of Ointments. The mice were anesthetized using lidocaine. The mice's fur around the back of the mice was shaved and swabbed with 70% alcohol. Then, an incision was made on the back of the mouse using a one mm long scalpel. Each incision was treated by applying mango leaf extract to the wound twice a day. The same procedure was performed using Bioplacenton Gel and Vaseline. The length of the wound was measured on days of 2, 4, 6, 8, 10, 12 and 14. These observations were created at 2-day intervals to evaluate physical changes of the wound area (Sumoza, 2014).

Results

The species of mango plant was stated as *Mangifera indica* L by the Director of the Laboratory of Mathematics and Natural Sciences, Universitas Andalas, Padang.

Tabel 1
Phytochemical Screening of Mangos Leaf Extract

Phytochemical	Reagents	Observation	Results
Alkaloid	HCL 2 N + Mayer	A yellow precipitate occurs	+
	HCL 2 + Wagner	A reddish precipitate occurs	+
	HCL 2 N + Dragendorff	An orange precipitate	+

Flavonoid	H ₂ SO ₄	occurs A reddish yellow color	+
Tannin	FeCl ₃	occurs A black color occurs Foam 1 cm- high occurs	+
Saponin	Hot water + HCL 2 N	A yellow color occurs	-
Terpenoid	Glacial CH ₃ COOH+ H ₂ SO ₄	A yellow color occurs	-
Steroid	Glacial CH ₃ COOH +H ₂ SO ₄		-

From the phytochemical screening test, it was found that the mango leaf extract was positive containing of flavonoid, saponin, tannin, and alkaloid compounds.

Tabel 2
The Average of Wound Healing
Reduced Length

Group	Average percentage of wound healing on day 14	Standard Deviation
Positive control	25.4	3.05
Negative control	11.4	1.14
Ointment 10%	14.4	2.07
Ointment 20 %	17.2	3.70
Ointment 40 %	16.8	4.15



Figure 1a and 1b.

Fresh and chopped mangos leave

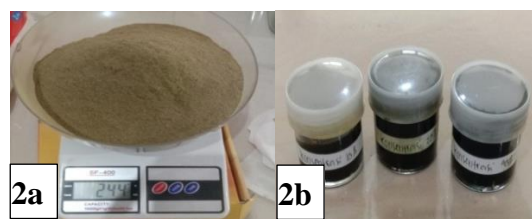


Figure 2a and 2b.

The powder of mango leaves and mangos leave extract ointments.

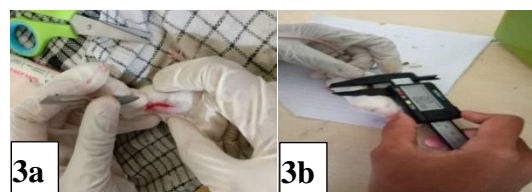


Figure 3a and 3 b.

Performing of cut wounds and measuring of wound length.

Pembahasan

It was observed that increasing of the concentration of mangos leave extract from 10 to 20 %, the decreasing of the length of wounds significantly. But not so much difference between 20 and 40 %. There was significant difference between negative control and 20 % ointment as well as 40 % ointment. The positive control seemed to give the very short wounds compare with all of another treatment. No significant difference between 20 and 40% of mangos leave extract. The wound that closed quickly after treatment with positive control. Each treatment group has a different percentage of wound healing. Starting from the negative control treatment group which had the longest wound compare to the treatment using ointment at the concentration of 40, 20, 10%, and positive control. It means all of formulation gave the shorter wound compare to the negative control.

Wound observations were carried out at 2-day intervals to determine physical changes in the wound area. The occurrence of swelling and granulation in the wound. In this phase the wound is filled with fibrous inflammatory cells, collagen fibers, new capillaries, forming

a reddish tissue with an uneven surface called granulation tissue. The rate of scab formation in each treatment group indicates the rate of wound healing.

The process of wound healing is physiologically divided into three phases, i.e the inflammatory phase, the proliferative phase, and the maturation phase. The inflammatory phase lasts from the onset of the wound until day 3. The first thing that happens after the wound is the activation of platelets. Damaged blood vessels in the wound will cause bleeding and the body will stop it by vasoconstriction, shrinking the ends of broken blood vessels and hemostatic reactions (Sjamsuhidajat, 2010).

The proliferative phase is also called the fibroplasia phase because the fibroblast cell proliferation process is very prominent. This phase lasts from day 3 to day 14 after the injury. The maturation phase occurs after the proliferative phase finish, around day 14 and can be up to 365 days after the injury occurs and is declared to end when all inflammation has disappeared. In this phase the body tries to return everything that becomes abnormal when the wound healing process becomes normal (Maryunani, 2015).

Based on the results of the ANOVA statistical test, the data on the percentage of wounds on day 14 obtained showed a significance value of $p < 0.05$, which means that there was a difference in the effect of giving positive control, and negative control with mango leaf extract.

Results of observations and data analysis showed that the shrinkage of the length of the open wound occurred faster and closed in the positive control group and at a concentration of 20 and 40% had the longer average percentage in wound healing. This demonstrated that there were differences in the impact of giving the mango leaf extract on wound healing.

This effect is due to the chemical content of tannins, saponins, and

flavonoids in mango leaves. The content of tannin compounds in the mango leaf extract is able to reduce tissue swelling and avoid the formation of pus on the wound surface due to pathogen invasion which can inhibit healing. The content of saponin compounds can increase the number of macrophages that migrate to the wound area, thereby increasing the production of cytokines that will activate fibroblasts in the wound tissue, then stimulate the formation of collagen which has a role in the wound healing process. The content of flavonoid compounds is believed to reduce the degree of erythema in wounds and inhibit the growth of bacteria (Liantari, 2014).

Conclusion

The mango leaf extract can cure second level cut wounds in male white mice. There was a difference in the effect of dose of mango leaf extract with a concentration of 20 and 40% on wound healing in male white mice compare to the negative control.

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