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Original Article

WOUND-HEALING TEST OF *PIPER BETLE* LEAF EXTRACT AND *ALOE VERA* IN GEL PREPARATION

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ABSTRACT

Objective: Burns are skin injuries primarily caused by heat, or due to electricity, chemicals, friction, radiation or fire. It is known that *Piper betle* and *Aloe vera* are able to accelerate the healing process of burns. The aim of this research was to evaluate the burn-healing activity of a *Piper betle* L. and *Aloe vera* combination in a gel preparation.

Methods: Carboxymethylcellulose (CMC) at various concentration was used in the formulation of gel. The formulation of the gel was evaluated for an organoleptic observation, measurement of pH, viscosity, and stability test. A burn-healing evaluation was performed *in vivo* by making a wound with a hot plate on a wistar rat male. Then 600 mg of gel was applied to the wound. After that, the presence of erythema, eschar, edema, and the extent of the burns were observed for 15 d after gel administration.

Results: The results showed that the best formulation of gel consisted of 2 % w/w of carboxymethyl cellulose (CMC) 1.1 % w/w of *Aloe vera* extract, and 3 % w/w of *Piper betle* extract. The gel preparation of *Piper betle* and *Aloe vera* extract could accelerate burn healing, where the healing percentage on the 9th d (53+1.3 %) is higher than the control (21+1.2%) as well as the erythema and eschar, which is lower than the control.

Conclusion: The gel preparation of the *Piper betle* and *Aloe vera* extract consisting of 2 % w/w, CMC 1.1 % w/w of *Aloe vera* extract, and 3 % w/w of *Piper betle* L. extract can accelerate burn healing.

Keywords: *Piper betle* leaf, *Aloe vera* gel, Burns, Gel

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INTRODUCTION

Burns are frequent injuries experienced by individuals in society. The burns are on the top rank of injuries suffered by human beings [1]. Burns are not only caused by direct and indirect fire contact but also by high-temperature exposure of the sun, electricity, chemical substances, light, or friction [2]. The level of severity of a burn depends on the condition of the burned skin tissue and intensity of the burn [3].

The factors affecting the burn-healing process are tissue repair and cell regeneration. Clinical conditions, such as infection, diabetes mellitus, or vitamin and protein deficiencies, can also affect the healing process. Proteins or amino acids and vitamins in the body can decrease inflammation and increase cell activity in the burn-healing process [4]. The final purpose of burn management is wound healing and epithelization in a short period of time in order to reduce functional and aesthetic after-effects and to prevent infection [5].

Piper betle belongs to the *Piperaceae* black pepper family. This plant probably originated from Malaysia [6]. *Piper betle* is one of the traditional plants that can increase wound healing process [7]. The saponin and phenol contents may be able to kill microorganisms on the burn wound and rash, as well as have the ability to stimulate the growth of skin cells [8]. A previous study has shown that an ethanol extract of betel can increase the healing of burns on an induced diabetic rat [9]. *Piper betle* are credited with many biological activities such as being a stimulant [10], a carminative, an expectorant [11], and an antiseptic [12], as well as the stopping of excessive bleeding during menstruation [13]. The scanning electron microscopy analysis confirmed that *Piper betle* L has the wound healing activity in rabbits [14]. Besides that *Piper betle* L has activity in cardiovascular, anti-inflammatory, hepatoprotective, antiulcer, immunomodulatory [15], and neuroprotective effects [16].

Aloe vera belongs to the Liliaceae family, which is a nutritional plant that has the ability to give protection to injured skin. Burn healing is one of the major indications of the *Aloe vera* gel [17]. The content of anthraquinone and saponin in *Aloe vera* performs as an analgesic and antiseptic agent [18]. In addition, the abundance of vitamins and proteins in *Aloe vera* provides a significant role in the burn-healing process [19]. *Aloe vera* influences collagen composition and increases collagen cross-linking for wound contraction and improving breaking strength [20]. It is reported that *Aloe vera* has a burn-healing activity on mice given *Aloe vera* extract for 14 d [21].

In the previous study, a large *Aloe vera* concentration was required to accelerate wound healing. A large concentration of *Aloe vera* caused the gel preparation was less stable. This study produced gel preparations with a small concentration from *Aloe vera* and combined with *Piper betle* to produce a stable gel preparation that is effective in wound healing [21]. The aim of this research was to evaluate the burn-healing activity of a *Piper betle* L. and *Aloe vera* combination in a gel preparation.

MATERIALS AND METHODS

Materials

Piper betle L. were purchased from the Research Institute for Medicinal Plants (BALITRO) Bandung Indonesia and were authenticated by School of Science and Biological Technology Institut Teknologi Bandung (ITB) with specimen no. 25. *Aloe vera* purchased from PT Dwipar Loka Ayu, Indonesia. Carboxy-methylcellulose (CMC), Methylparaben, Propylparaben, propylene glycol was purchased from PT Brataco Chemical, Indonesia. Wistar strain white rat was obtained from ITB.

Methods

Phytochemical screening

Phytochemical screening of *Piper betle* and *Aloe vera* extracts was carried out for the detection of secondary metabolites such as tannins, alkaloids, flavonoids, terpenoids, steroids, and saponins [6].

Formulation of gel

Gel preparations were made with various concentrations of CMC and *Aloe vera* extracts. There were six formulas consisting of three

different concentrations of CMC and two different concentrations of *Aloe vera* extract [21].

Table 1: Composition of gel preparation

Composition	Concentration (% w/w)					
	F1	F2	F3	F4	F5	F6
Piper betle Extract	3.00	3.00	3.00	3.00	3.00	3.00
Aloe vera extract	1.10	1.30	1.10	1.30	1.10	1.30
CMC	2.00	2.00	3.00	3.00	4.00	4.00
Propylene glycol	10.00	10.00	10.00	10.00	10.00	10.00
Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
Propyl paraben	0.01	0.01	0.01	0.01	0.01	0.01
Water ad	100	100	100	100	100	100

CMC was dissolved in water until the gel base formed, and then propylene glycol was added. Bettle leaf extract, *Aloe vera* extract, methylparaben, and propylparaben were dissolved into water, added into the base gel mixture, and stirred until a homogeneous mass was obtained. Water was added until the gel mass was 100 g.

Organoleptic examination

Organoleptic properties of the gel preparations were observed for every change such as consistency, color, and odor at room temperature on the 1st, 3rd, 7th, 14th, 21st, and 28th d.

pH measurement

Measurement of pH of the gel preparations was performed by pH meter on the 1st, 3rd, 7th, 14th, 21st, and 28th d.

Stability test

A stability test was performed by observing any change to consistency, color, and odor in gel preparation placed at room temperature (27 °C), cool temperature (10 °C), and in open container on the 1st, 3rd, 7th, 14th, 21st, and 28th d.

Viscosity measurement

The viscosity of gel preparations was measured by Brookfield Viscometer on 1st, 3rd, 7th, 14th, 21st, and 28th d.

***In vivo* burn-healing test**

The ethical approval for the experimental procedure for the irritation test was obtained from the Health Research Ethics Committee, Faculty of Medicine, University of Padjadjaran (KEPK-FK UNPAD). Twelve rats (n for each treatment was three rats) used in this research were shaved in the back. The burns were created by a hot plate (diameter 1 cm) on 80 °C for 5 s. The burn-healing process was determined by the extension of wound measurement and presence of erythema and eschar before and after the treatment was applied [22, 23].

Table 2: *In vivo* test of burn wounds

Part of wound	I	II	III	IV
Treatment	Negative control (Physiological water)	Base control	*Piper betle* and *Aloe vera* extract	Formulation of gel preparation

Statistical analysis

The data of the experiments are presented as a mean of samples±standard deviation (SD) and were analyzed using the one way analysis of variance (ANOVA) at the level of ($P<0.05$) to determine if the changes in the applied factors are statistically significant at level of ($p<0.05$) and non-significant at level of ($p>0.05$).

RESULTS AND DISCUSSION

The species of the *Piper betle* used in this research is *Piper betle* L. [6], while the species of *Aloe vera* is *Aloe barbadensis* Mill [24].

Phytochemical screening

Phytochemical screening was conducted to find the secondary metabolite contained in *Piper betle* and *Aloe vera* extract. The result of phytochemical screening was *Piper betle* extract contained saponin, tannin, quinone, and monosesquiterpene, while the powder of *Aloe vera* contained saponin. Polyphenol could accelerate tissue regeneration and protect it from infection (antiseptic agent).

It was used as an astringent and healing agent. The astringent property is responsible for increasing rates of epithelization and wound contraction [25]. Due to antimicrobial and antioxidant properties of tannin, it could be used to promote the wound healing process [26]. By promoting capillary vasoconstriction, tannin acts as a local anti-inflammatory agent to decrease vascular permeability [27].

Saponin is a non-volatile compound that is widely spread in all plant species, the most common species are from the Liliaceae family. It is reported that saponin could act as an antioxidant and antimicrobial agent; those properties are responsible for wound contraction and the high rate of epithelization [28]. Literature has reported that polysaccharides in *Aloe vera* act as immunostimulants. It enhances the release of cytokines, which are indirectly responsible for wound healing. *Aloe vera* also increases collagen activity, which leads to wound contraction [29]. This complex carbohydrate from Aloe vera can accelerate wound healing and can reduce radiation-induced skin reactions [20].

Formulation of gel

The gel base used in this study was a derivative of cellulose, CMC, with various concentrations, namely 2 % w/w, 3 % w/w, and 4 % w/w. Carbomers could not be used as a gel base because there was an incompatibility between carbomer and polyphenol contents of the *Piper betle* extract. Carbomers are incompatible with cationic polymers, phenol, strong acids, and a high level of electrolytes [30].

Propylene glycol in this formulation was used as a moisturizer and enhancer. The enhancer mechanism of propylene glycol increased the solubility of the active substance in the stratum corneum by transiently changing the solubility parameter of the skin. Consequently, there may be an improvement in drug diffusion through the skin [31].

Table 3: Stability test result of *Piper betle* and *Aloe vera* extract

Formula	Evaluation	D					
		1	3	7	14	21	28
1	Color	Green brownish					
	Odor	Typical aromatic odor					
	Consistency	***	***	***	***	***	***
2	Color	Green brownish					
	Odor	Typical aromatic odor					
	Consistency	***	***	***	***	***	***
3	Color	Green brownish					
	Odor	Typical aromatic odor					
	Consistency	*	*	*	*	*	*
4	Color	Green brownish					
	Odor	Typical aromatic odor					
	Consistency	*	*	*	*	*	*
5	Color	Green brownish					
	Odor	Typical aromatic odor					
	Consistency	**	**	**	**	**	**
6	Color	Green brownish					
	Odor	Typical aromatic odor					
	Consistency	**	**	**	**	**	**

Notes: *: thick, **: very thick, ***: semi-fluid

Based on the results above, there were differences in consistency in each formula due to the amount of gel base used. The higher base concentration used, the higher consistency of gel preparation obtained. All of the gel preparations were stable in consistency, odor, and color after stored for a certain period of time. The best formula based on organoleptic examination was thefirst formula (F1).

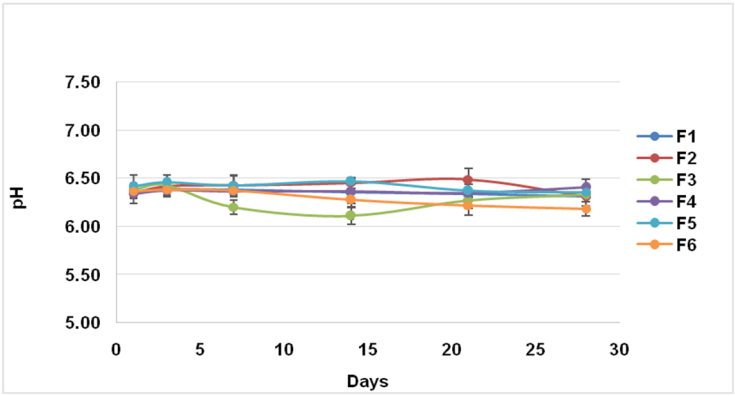

Fig. 1: pH measurement result of *Piper betle* and *Aloe vera* extract gel (All the values were calculated as mean±standard deviation; n=3)

The pH measurement results were analyzed statistically and showed that ρ value was more than 0.05, so H_0 was accepted and H_a was rejected, which means that there was no significant difference between pH in each formula during storage time.

Piper betle and *Aloe vera* extract gel are aimed to skin dosage form, so the preparation of pH should be appropriate for skin pH (4,5–6,5). The results showed that the pH of gel formulations was in the range of 6-6.5, which lies in the normal pH range of the skin. It is known that the pH affected the penetration rate of the preparation, dissociation level, the solubility of lipophilic drug, and viscosity stability of the gel preparation [32]. Based on the graph, the most stable formula on pH during storage time was the first and third formulas because there was no significant decline of pH among those formulas.

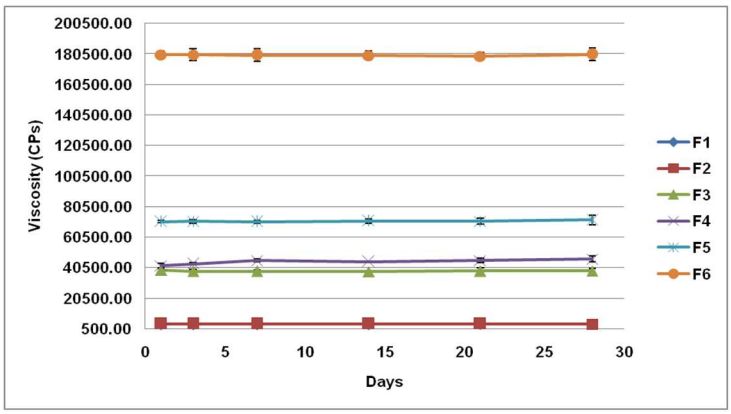

Fig. 2: Viscosity of *Piper betle* L. and *Aloe vera* extract gel (All the values were calculated as mean±standard deviation; n=3)

Fig. 2 showed that a higher concentration of CMC resulted in higher viscosity. According to Pednekar *et al.*, increased polymer concentration can increase the viscosity of gel [33]. The viscosity measurement results were analyzed statistically, and showed that the ρ value is more than 0.05; therefore, H_0 was accepted and H_a was rejected, which means that there was no significant difference between the viscosity in each formula and storage time.

The result of the stability test of *Piper betle* and *Aloe vera* extract at a cool temperature was the same, as well as the stability test result of the gel at room temperature—that is, the preparation was still stable until the end of storage time. Meanwhile, stability in an open container showed that the gel had changed during storage time, as the gel changed into a more rigid form. This might have been caused by direct contact between the gel and air; therefore, syneresis and water loss occurred [34].

***In vivo* burn-healing test**

The parameter used for the burn-healing test was the percentage of the wound healing. The percentage was obtained from the wound diameter of each treatment, and then calculated with the following equation:

$$\% \text{ percentage of wound healing} = \frac{100\ (A_o - A_t)}{A_o}$$

A_o is the initial wound diameter, and A_t is the wound diameter on d t. After that percentage of the wound was analyzed statistically.

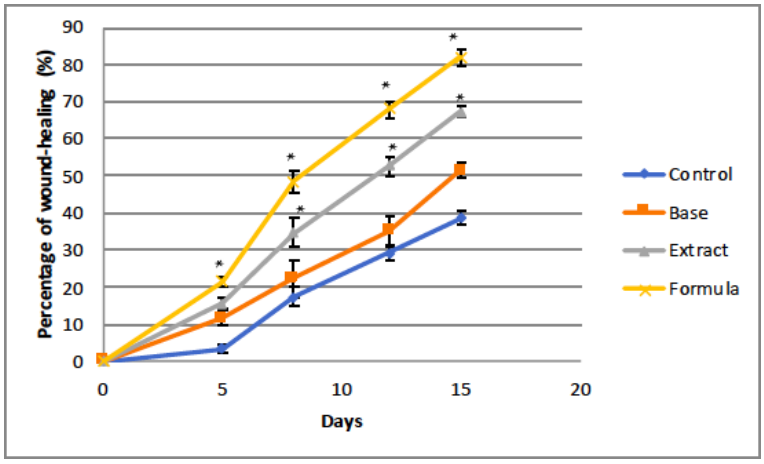

Fig. 3: The result of wound-healing percentage (All the values were calculated as mean±standard deviation; n=3 and statistically significant results are given as * P<0.05 compared to control

The average wound diameter on the Wistar rat was analyzed statistically and showed that ρ value was less than 0,05; therefore, H_0 was rejected and H_a was accepted, which means that there were significant differences among each treatment (formula group versus control, base, and extract group). On the 9th d, the percentage of the wound healing of the formula group was 53+1.3 %, which means it was greater than control with the percentage of 21+1.2 % wound healing.

Moist conditions on the wound would accelerate epithelization of the wound. Dressing the wound with hydrogel bandage can stabilize the moisture of the tissue and optimize ephitelization [18]. The content in *Aloe vera* and *Piper betle* can accelerate wound healing such as vitamin A, which is required for epithelial and bone formation; Vitamin C, which is necessary for collagen formation; Vitamin E, which is the major lipid-soluble antioxidant in the skin and amino acids may influence wound repair and immune function [4].

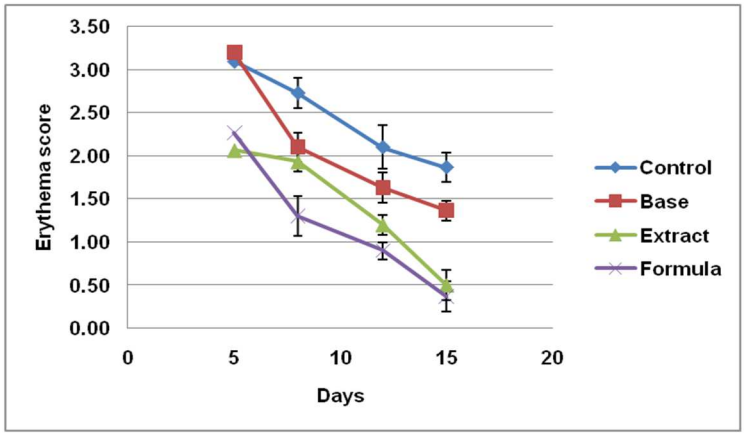

Fig. 4: Erythema observation results (All the values were calculated as mean±standard deviation; n=3)

Based on the fig. above, it is apparent that *Piper betle* and *Aloe vera* extract treatment can reduce erythema better than other treatments. It might be caused by the saponin content in the *Piper betle* and *Aloe vera* extract as well as the water content in the gel preparation. Thus, inflammation can be reduced [28]. Eschar is a layer of dead skin tissue occurring, in particular, after burn injuries. It forms a hard surface that can block blood flow in those areas, causing the wound closing process (re-epithelization) to transpire imperfectly. On eschar observation, *Piper betle* and *Aloe vera* extract gel groups have lower scores than the control group. This shows that the *Piper betle* and *Aloe vera* gel could accelerate the wound healing process [21].

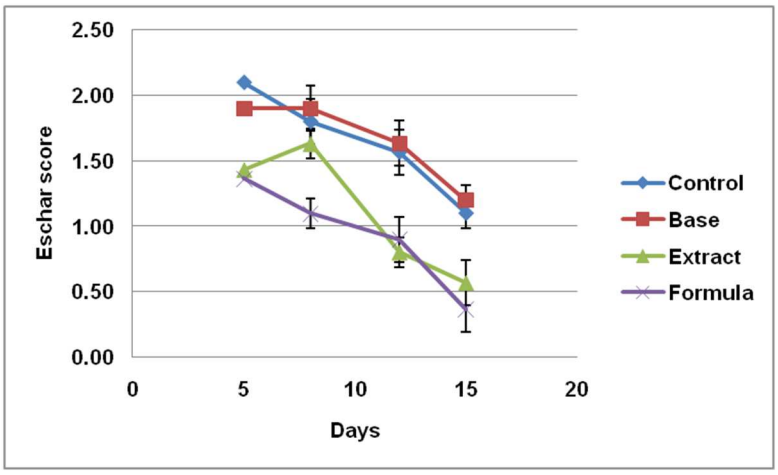

Fig. 5: Eschar observation result (All the values were calculated as mean±standard deviation; n=3)

CONCLUSION

Based on stability evaluation, the best gel preparation was the formula containing 2% w/w CMC, 1,1% w/w *Aloe vera* extract, and 3% w/w *Piper betle* extract. *Piper betle* and *Aloe vera* extract gel could accelerate the burn-healing process with a higher percentage of wound-healing apparent on the 9th d, where the formula group percentage was 53+1.3 %, and the control group was 21+1.2 %. Also the erythema, eschar and edema scores of the formula group that d were lower that the control group.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

All authors have none to declare

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