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THE EFFECT OF FORMULA TO PHYSICAL PROPERTIES OF WOUND HEALING GEL OF ETHANOLIC EXTRACT OF BINAHONG (*ANREDERA CORDIFOLIA* (TEN) STEENIS)

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ABSTRACT

Binahong (*Anredera cordifolia* (Ten) Steenis) has been used as wound healing in traditional Indonesian medicine and it is relevant to develop dosage forms of binahong using formulation technology approach. The aim of this research was to find out wound healing gel formula of ethanolic extract of binahong. The factorial design method 3 factors and 2 levels were employed to achieve this study. Three factors used in this study were Carbopol, Na-CMC and Ca-alginate with low and high level for each factor. The wound healing gel of ethanolic extract of binahong was evaluated for their physical properties i.e viscosity, spreadability, extrudability and bioadhesive properties. The results of this research were carbopol and Ca-alginate affecting physical properties of wound healing gel of ethanolic extract of binahong. Na-CMC affected physical properties of the gel except bioadhesive properties. Carbopol provided the greatest contribution to viscosity, spreadability, extrudability and bioadhesive properties of the gel.

INTRODUCTION: Binahong (*Anredera cordifolia* (Ten) Steenis) has been used as wound healing in traditional Indonesian medicine. The leaf of binahong was crushed and put in the wound site. Chemical investigation of binahong found six sapogenin, one of them was ursolic acid [1]. Ursolic acid stimulated epidermal keratinocyte differentiation via peroxisome proliferator-activated receptor-α [2]. It is relevant to develop wound healing dosage forms of binahong using formulation technology appoach.

The primary goal of wound management is to heal the wound in the shortest time with minimal pain, discomfort and scarring to the patient [3]. The wound healing dosage forms should provide physiological environment to repairing and regenerating process of the wound site. It should give moist environment.

The benefits of the moist environment are preventing the tissue dehidration and cell death, accelerating the angiogenesis and increasing the breakdown of death tissue and fibrin. Gel is appropriate as wound healing dosage forms [4].

The composition of gel base will affect its physical properties. Hydrogel for dermatology has several properties such as greaseless, tixotrophy, easy to spread, easy to remove and emollient [5]. Carbopol is used as gel base in the range of 0.5-2.0% [4]. Sodium carboxymethylcellulose (Na-CMC) will produce smooth and elastic gel in the range of 3.0-6.0% of the solution. Calcium alginate is used as gel base with minimal concentration 0.5%. Gelling properties of Ca-alginate, highly absorbent, is suitable for exuding wounds [6, 7].

Carbopol, Na-CMC and Ca-alginate blend impart sufficient viscosity to provide moisture to the wound site [8]. The physical properties of the gel is affected by concentration of gelling agent in the solution [9].

MATERIALS AND METHODS: The materials used in this research was: dry leaves of binahong, ethanol, carbopol, sodium carboxymethylcellulose, calcium alginates, glycerol, triethanolamine, boric acid, potassium sorbate. All of the materials were of pharmaceutical grade.

Extraction of dry leaves of binahong. Twenty gram dry leaves of binahong were weighed, powdered and macerated with 200 mL ethanol for 90 minutes, at temperature 50°C and shaked at 200 rpm. The maserat was separated and concentrated until 25% of volume and it was called ethanolic extract of binahong.

Preparation of wound healing gel of ethanolic extract of binahong.

Standard formula:

1. R/Carbopol 940: 1,0
2. Na-CMC : 0,6
3. Ca-alginate : 0,75
4. Trietanolamine: 3 (until pH 7)
5. Glycerol : 12,5
6. Boric acid : 0,5
7. K sorbate : 0,2
8. Ethanolic extract of binahong: 5 ml
9. Water : q.s upto 100

Na-CMC was poured into water and stirred with mixer for 10 minutes and 400 rpm, then added by Ca-alginate, stirred again for 10 minutes and 400 rpm, finally Carbopol was added into the mixture. It was all stirred until homogeneous. Boric acid and potassium sorbate that have been dissolved in water, were added to the mixture above. Glycerol was added into the mixture then was stirred until homogeneous. Finally, triethanolamine was incoporated into the mixture then it was stirred gently until pH 7. This mixture was gel base. The gel base was sterilized by autoclave at 115°C for 30 minutes. The sterilized gel base was transferred to the laminair air flow. Under laminair air flow condition, ethanolic extract of binahong was incorporated to the sterilized gel base. The experiment was directed by design factorial as indicated in **Table 1**.

TABLE 1: A 2^3 FULL FACTORIAL DESIGN IN THE DEVELOPMENT OF WOUND HEALING GEL FORMULA OF ETHANOLIC EXTRACT OF BINAHONG

Variable	Factors	Coded level	
		-1	1
A	Carbopol (g)	0,75	1,25
B	Na-CMC (g)	0,2	0,8
C	Ca-alginate (g)	0,5	1,5

Determination of Physical Properties:

1. **Viscosity determination**. Wound healing gel of ethanolic extract of binahong was put into a container. Then, the portable viscometer was put in the container. The viscosity was obtained by monitoring the moving of the viscosity pointer.

2. **Spreadability determination**. Two grams of wound healing gel of ethanolic extract of binahong was put in the middle of the ground glass slide. The gel was sandwiched between two ground glass slides. One kg of burden was placed on the top of the two slides for 3 minutes. The top slide was subjected to pull of the 80 g. The time and the distance required to separate the two slides were noted. The spreadability was then calculated by the following formula;

$$S = M \times L / T.$$

Where, S = speadability, M = the weight in the pan (tied to the upper slide), L = the length moved by the glass slide, and T = represents the time to separate the slide completely from each other.

3. **Extrudability study. The** modified of Kumar and Verma (2010) [10] method was used to measure extrudability properties of the gel. This test was used to determine the force required to extrude gel from the tube. The gel was inserted into the aluminium tube. The tube was forced with 1 kg for 10 second. The gel that was extruded from the tube was accommodated into the water fully contained bekker glass. The dropped water was weighed. The extruded gel volume was measured based on the spilled water.

4. **Bioadhesion study**. The modified of Wittaya-areekul *et al*., (2005) [11] and Kumar and Verma (2010) [10] method was used to measure bioadhesive properties of the gel. The pig's large

intestine was used to represent the mucous-like texture of a fresh wound. Freshly slaughtered pig's large intestine was washed with physiological saline. Then, it was attached to a platform. 0,5 g of the gel was sandwiched between two platforms, and held under 100 g weight for 1 minutes. The other side of platform was connected to a pulley system. The bioadhesion was measured by adding water to container on the pulley system. The weight of water that was required to detach the platform was recorded. The bioadhesive strength was calculated based on equation:

$$B = W/A$$

Where, B = bioadhesive strength (g/cm^2), W = weight of water required to detach the platform (g), A = area (cm^2)

RESULTS AND DISCUSSION: The physical properties of wound healing gel of ethanolic extract of binahong was done to represent the acceptibility of consumers. The consumers pretended to use product based on the texture profile of product, i.e., appearance, odor, extrudability, initial sensation, spreadability, and smoothless of the dosage forms

The formula of wound healing gel of ethanolic extract of binahong that contained large number of carbopol provided high viscosity, low spreadability, low extrudability and high bioadhesive properties. The wound healing gel of ethanolic extract of binahong that contained large number of Na-CMC provided low viscosity, high spreadability, high extrudability and low bioadhesive properties, and so did the gel that contained large number of Ca-alginate (**Table 2**).

Carbopol provided the largest contribution to all of the physical properties of the wound healing gel of ethanolic extract of binahong, on the other hand Ca-alginate provided the smallest contribution (**Tabel 3**). The negative value meant that the increasing of gelling agent added in the solusion would decrease the physical properties of the gel. The positive value meant that the increasing of gelling agent added in the solution would increase the physical properties of the gel.

TABLE 2: VISCOSITY, SPREADABILITY, EXTRUDABILITY AND BIOADHESIVE PROPERTIES OF WOUND HEALING GEL OF ETHANOLIC EXTRACT OF BINAHONG

Formula	A (g)	B (g)	C (g)	Viscosity (d.PaS)	Spreadability (g.cm/s)	Extrudability (cm^3)	Bioadhesion (g/cm^2)
1	0,75	0,20	0,50	52,08 ± 2,46	106,99 ± 10,70	7,46 ± 0,17	15,28 ± 0,59
a	1,25	0,80	0,50	170,00 ± 8,37	32,31 ± 1,78	0.995 ± 0,09	20,36 ± 0,43
b	0,75	0,20	0,50	88,33 ± 5,16	69,98 ± 2,78	5,55 ± 0,15	15,19 ± 0,30
ab	1,25	0,80	0,50	168,33 ± 9,31	30, 75 ± 2,37	0,91 ± 0,07	17, 01 ± 0,32
c	0,75	0,20	1,50	84,17 ± 2,58	110,62 ± 5,61	4,69 ± 0,25	14,01 ± 0,44
ac	1,25	0,80	1,50	163,33 ± 9,83	42,08 ± 1,75	1,74 ± 0,17	16,18 ± 0,43
bc	0,75	0,20	1,50	123,33 ± 4,08	44,46 ± 1,95	4,96 ± 0,12	13,50 ± 0,39
abc	1,25	0,80	1,50	182,50 ± 6,12	19,88 ± 1,45	1,02 ± 0,10	20,93 ± 0,74

TABLE 3: CONTRIBUTION OF CARBOPOL, Na-CMC AND Ca-ALGINATE TO PHYSICAL PROPERTIES OF WOUND HEALING GEL OF ETHANOLIC EXTRACT OF BINAHONG

	Viscosity	Spreadability	Extrudability	Bioadhesion
Carbopol (A)	84,08	-52,92	-4,51	4,12
Na-CMC (B)	23,23	-32,90	-0,61	0,20
Ca-alginate (C)	18,65	-4,58	-0,63	-0,80
AB	-14,48	18,69	0,21	0,50
AC	-14,90	6,37	1,06	0,67
BC	5,94	-11,28	0,38	1,97
ABC	4,48	3,29	-0,71	2,13

The appropriate viscosity of gel dosage forms would help patient apply it during administration. The viscous gel would make it difficult to apply, neither did the liquid one. The design factorial would show which parameter (Carbopol, Na-CMC or Ca-alginate) affected the most to viscosity properties of wound healing gel of ethanolic extract of binahong. Carbopol, Na-CMC and Ca-alginate provided positif contribution to the viscosity properties of the gel (**Table 3**). It meant that the more the Carbopol, Na-CMC or Ca-alginate was added, the more viscous the gel. Carbopol swelled in the water to form irregular fibrous network stucture. The increasing carbopol in the system would increase cross-link density then it increased the viscosity of system. Gel containing 1% of carbopol would form typical network structure as honeycomb structure in the presence of triethanolamine. This structure was stronger than irregular fibrous network structure [12]. The increasing carbopol in the gel system would enhance honeycomb structure then it increased the viscosity properties of the gel system. It caused the greatest contribution of carbopol among other gelling agent.

The viscosity and structure rearrangement of Na-CMC in the water depended on the degree of its neutralization. In the water, Na was released and changed with H to form HCMC, then the cross-lingking occured and the solusion viscosity increased [13]. Hidrogen bonding decreased the solubility of Na-CMC in the water and formed elastic hydrogel formation. The increasing Na-CMC in the solution resulted the increasing hidrogen bonding and the rigidity of the solution. It caused positif contribution of Na-CMC to the viscosity properties.

The alginate gellation was formed by affinity of alginate toward certain ions and its ability to bind these ions selectively and cooperatively. The increasing ionic binding would enhance the viscosity of the gel. Ca^{2+} was strictly binding guluronate residues (G), so the alginate rich of G-blocks would be more viscous than the alginate rich of manuronate residues (M) [14]. The viscosity of alginate gel depended on ionic strength of the solvent and alginate concentration in the solution [15]. The increasing Ca-alginate concentration in the solution would enhance the viscosity of the wound healing gel of ethanolic extract of binahong.

The interaction between carbopol with other gelling agent (Na-CMC and Ca-alginate) would lower the viscosity, while interaction between Na-CMC and Ca-alginate would enhance the viscosity of the gel (**Table 3**). This phenomenon was interesting. Na-CMC and Ca-alginate could interact with monovalent and polivalent ion through ionic binding. So, interaction between Na-CMC and Ca-alginate would enhance the viscosity of the gel. The gellation of carbopol was caused by repulsion force of negative charge of its chains. The presence of Na^+ and Ca^{2+} would disturb the equilibrium and reduce the viscosity of the gel.

The spreading properties of the gel would affect the dose of active ingredient that was delivered to target site. The spreadability was the ability of the gel to spread in the site of application. The more viscous the gel, the more difficult to spread it. Carbopol, Na-CMC and Ca-alginate provided the significant contribution to spreading properties of the wound healing gel of ethanolic extract of binahong (**Table 4**). The negatif value of contributions of carbopol, Na-CMC and Ca-alginat showed that the more gelling agent was added in the solution the more difficult the gel to spread.

TABLE 4: RESULTS OF REGRESSION ANALYSIS (ANOVA) FOR VISCOSITY, SPREADABILITY, EXTRUDABILITY AND BIOADHESION PROPERTIES OF WOUND HEALING GEL OF ETHANOLIC EXTRACT OF BINAHONG

Source	Prob > F			
	Viscosity	Spreadability	Extrudability	Bioadhesion
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Carbopol (A)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Na-CMC (B)	< 0.0001	< 0.0001	< 0.0001	0.1448
Ca-alginate (C)	< 0.0001	0.0117	< 0.0001	< 0.0001
AB	< 0.0001	< 0.0001	< 0.0001	0.0008
AC	< 0.0001	0.0007	< 0.0001	< 0.0001
BC	0.0033	< 0.0001	< 0.0001	< 0.0001
ABC	0.0234	0.0650	< 0.0001	< 0.0001

Note : significant if Prob>F has a value less than 0,05.

The extrudability of gel was the parameter to measure energy that was used to extrude the gel out of the tube. If the gel was very viscous, the gel would be difficult to extrude out of the tube. It certainly reduced the patient's convenience. In this study, parameter of extrudability was not the energy needed to extrude the gel out of the tube.

Parameters measured were the volume of gel that came out of the tube due to application of 1 kg load for 10 seconds given to the tube. All of gelling agent provided the significant contribution to extrudability properties of wound healing gel of ethanolic extract of binahong (**Table 4**). Carbopol provided the greatest contribution in the extrudability properties (**Table 3**).

The increasing carbopol concentration in the solution would decrease the extrudability of the wound healing gel of ethanolic extract of binahong. In other word, the increasing carbopol concentration would enhance the energy needed to extrude gel out of the tube. Honeycomb structure of the carbopol solution caused it difficult to flow out of the tube, it required the large energy to extrude it. These phenomenon explained the greatest contribution of carbopol to the extrudability properties of the wound healing gel of ethanolic extract of binahong compared to its of Na-CMC and Ca-alginate.

The contribution of Na-CMC and Ca-alginate to extrudability properties was similar. Both of gelling agent formed a gel with physical cross-linking and it took less energy to flow out of the tube. While on carbopol, bonding formation occured through chemical cross-linking. Chemical cross-linking was stronger than physical cross-linking, so that the bond was stonger, the gel was more rigid, and there was more energy required to extrude gel out of the tube. This explained that the contribution both of Na-CMC and Ca-alginate were smaller than carbopol (**Table 3**).

The term bioadhesion was used to describe a phenomenon that was associated with the capacity of macromolecule to adhere to biological tissue in the presence of water [16]. The bioadhesioan determined how long the gel would adhere to the skin. The wound healing gel of ethanolic extract of binahong might be able to adhere to the wound site in sufficient time in order to release the active ingredient.

Carbopol and Ca-alginate provided the significant contribution to the bioadhesive properties of the wound healing gel of ethanolic extract of binahong. The increasing carbopol would enhance bioadhesion of wound healing gel of ethanolic extract of binahong, otherwise the increasing Ca-alginate would decrease bioadhesion of the gel (**Table 4**).

The bioadhesion was influenced by the material properties. The material properties required in order to have adhesive capacity were functional groups able to form hydrogen brigdes (-OH, -COOH), anionic charge, elasticity of the polymer chains to penetrate the mucus layer, and high molecular weight [16].

Carbopol provided the greatest contribution in the bioadhesive properties of the wound healing gel of ethanolic extract of binahong (**Table 3**). Carbopol would be adsorbed in the surface, decrease the surface free energy and then create a bond with surface layer [17]. Carbopol had a large number of groups which had capability to form hydrogen bridges with the surface of the mucus layer. The increasing carbopol in the solution would enhance free –COOH group which formed hydrogen bonding with the surface, so that it enhanced bioadhesive properties of wound healing gel of ethanolic extract of binahong.

Na-CMC provided no significant contribution in bioadhesive properties (**Table 4**). In the neutral solution, Na-CMC tended to release Na^+ and became negatively charged. The positive charge of cationic polymer would bind the wound surface which had negative charge. The anionic polymer had less capability to bind the wound surface compared with the cationic one. So, it was understandable that Na-CMC did not affect the bioadhesive properties of wound healing gel of ethanolic extract of binahong.

Ca-alginate contributed to bioadhesive properties of wound healing gel of ethanolic extract of binahong (**Table 4**). The increasing Ca-alginate concentration would decrease bioadhesion of wound healing gel to the surface of wound site. In the solution, the groups which had adhesive properties formed an egg box structure and interacted with carbopol and Na-CMC. The egg box structure was a helical conformation of alginate chain that enabled it to form gel.

This concluded that the increase of Ca-alginate would decrease bioadhesion of wound healing gel of ethanolic extract of binahong.

CONCLUSION: Carbopol and Ca-alginate affected physical properties of wound healing gel of ethanolic extract of binahong. Na-CMC affected physical properties of the gel except bioadhesive properties.

Carbopol provided the greatest contribution to viscosity, spreadability, extrudability and bioadhesive properties of the gel.

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