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Research Article

PHYSICAL PROPERTIES OF WOUND HEALING GEL OF ETHANOLIC EXTRACT OF BINAHONG (*Anredera cordifolia* (Ten) Steenis) DURING STORAGE.

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ABSTRACT

Binahong (*Anredera cordifolia* (Ten) Steenis) has been used as wound healing in traditional Indonesian medicine. The developing of the dosage forms using the formulation technology approach has been done. The aim of this study was to examine the physical properties of the wound healing gel of ethanolic extract of binahong during storage. The factorial design method 3 factors and 2 levels was employed to achieve this study. The three factors used in this study were Carbopol, CMC-Na and Ca-alginate with low and high level for each factor. The physical properties of the wound healing gel of ethanolic extract of binahong was evaluated on day 1, 30, 60 and 90 for viscosity and bioadhesion study. The result showed that CMC-Na and Ca-alginate increased the alteration of physical properties of wound healing gel of ethanolic extract of binahong during storage. The carbopol maintained the physical properties of the gel during storage.

Key words: bioadhesion, binahong, viscosity, wound healing gel.

INTRODUCTION

Binahong (*Anredera cordifolia* (Ten) Steenis) has been used as wound healing in traditional Indonesian medicine. The leaves of binahong were crushed and put in the wound site. The chemical investigation of binahong found six sapogenin, one of them was ursolic acid (Lin *et al.,* 1988). The ursolic acid stimulated epidermal keratinocyte differentiation via peroxisome proliferator-activated receptor-α (Lim *et al.,* 2007). It is relevant to develop wound healing dosage forms of binahong using formulation technology approach.

The primary goal of wound management is to heal the wound in the shortest time with minimal pain, discomfort and scarring to the patient (Cockbill, 2002). The wound healing dosage forms should provide physiological environment to repairing and regenerating process of the wound site. The gel is appropriate as wound healing dosage forms (Mallefet and Dweck, 2008). It gives moist environment which is preventing the tissue dehydration and cell death, accelerating the angiogenesis and increasing the breakdown of death tissue and fibrin (Mallefet and Dweck, 2008).

The unstable gel would exhibit some irreversible change in its rheological properties (Zatz and Kushla, 1996). The hydrogel degradation occurs due to hydrolysis and enzimatic reactions. Carbopol, CMC-Na and Ca-alginate as gelling agent are polymer that contain carboxylic group in their backbone. The presence of water will cause hydrolysis reaction of this polymer. The hydrolysis may occur either in the cross-link or the polymer backbone (Meyvis *et al.,* 2000). The reducing cross-link in the polymer backbone of gelling agent will reduce the viscosity of the gel (Martens *et al.,* 2001).

This study will examine the effect of the polymer to physical properties of the wound healing gel of the ethanolic extract of binahong during storage. The gel was packed in the tube and stored at temperature 40°C, RH 75% for 90 days.

METHODOLOGY

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

Methods

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

Preparation of wound healing gel of ethanolic extract of binahong.

Standar formula(Cornell *et al.,* 1997):

R/	Carbopol 940	1.0
	CMC-Na	0.6
	Ca-alginate	0.7
	Trietanolamine	3 (until pH 7)
	Glycerol	12.5
	Boric acid	0.5
	Potassium sorbate	0.2
	Ethanolic extract of binahong	5mL
	Water to	100

CMC-Na was poured into water and stirred with mixer for 10 minutes and 400rpm, then added by Ca-alginate, stirred again for 10 minutes and 400rpm. Finally, Carbopol was added into the mixture. They were all stirred until homogeneous. Solution of boric acid and potassium sorbate, were added to the mixture above. Glycerol was then added and stirred to homogeneous. Finally, triethanolamine was incorporated into the mixture then it was stirred gently and adjust the pH 7. This mixture was housed gel base. The gel base was sterilized by autoclave at 115°C for 30 minutes. The sterilized gel base was transferred to the laminar air flow. Under the laminar air flow condition, ethanolic extract of binahong was incorporated to the sterilized gel base. The gel was packed into the tube under LAF. The tubes were then kept in the climatic chamber at 40°C and RH of 75%. The experiment was directed by design factorial as indicated in table I.

Determination of physical properties during storage.

The physical properties was determined on the day 1, 30, 60 and 90 for viscosity and bioadhesion study.

Viscosity determination

The 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.

Bioadhesion study

The modified of Wittaya-areekul *et al.,* (2006) and Kumar and Verma (2010) method was applied to measure bioadhesive properties of the gel. The pig's large intestine was used to represent the mucous-like texture of a fresh wound. The freshly slaughtered pig's large intestine was washed with physiological saline. Then, it was attached to a platform, 0.5g of the gel was sandwiched between two platforms, and held under 100g 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 degradability of the hydrogel was based on the hydrolysis of the cross-link or polymer backbone. The hydrolysis of the polymer backbone can be achieved with the introducing of degradable unit of polymer backbone to the suitable enzyme (Meyvis *et al.,* 2000). The hydrolysis of the cross-link can occur with the presence of water.

The viscosity of wound healing gel of ethanolic extract of binahong tends to decrease during storage. The alteration of the viscosity of the gel may vary between 15 to 61% (Table II).

Table I. A 2^3 full factorial design in the development of wound healing gel formula of ethanolic extract of binahong.

Variable	Factors	Coded level	
		Low level	High level
A	Carbopol (g)	0.75	1.25
B	CMC-Na (g)	0.2	0.8
C	Ca-alginate (g)	0.5	1.5

Table II. The viscosity of the wound healing gel of ethanolic extract of binahong (*Anredera cordifolia* (Ten) Steenis) during storage.

For-mula	Factors			Time of storage				% Δ viscosity
	A (g)	B (g)	C (g)	Day 0 (d.PaS)	Day 30 (d.PaS)	Day 60 (d.PaS)	Day 90 (d.PaS)	
1	0.75	0.2	0.5	52.08±2.46	49.17±1.29	46.17±1.13	42.75±1.72	17.92±3.31
a	1.25	0.2	0.5	170.00±8.37	168.33±9.31	147.50±4.18	144.17±3.76	15.20±2.21
b	0.75	0.8	0.5	88.33±5.16	85.00±4.47	68.33±4.08	64.17±4.92	27.36±5.57
ab	1.25	0.8	0.5	168.33±9.31	160.00±5.48	156.67±8.16	133.33±4.08	20.79±2.43
c	0.75	0.2	1.5	84.17±2.58	76.67±2.58	58.33±2.58	43.83±0.98	47.92±1.17
ac	1.25	0.2	1.5	163.33±9.83	172.50±2.74	134.17±6.65	93.75±3.79	42.60±2.32
bc	0.75	0.8	1.5	123.33±4.08	124.17±3.76	83.33±5.16	47.92±1.07	61.15±0.87
abc	1.25	0.8	1.5	182.50±6.12	187.50±4.18	133.33±10.33	86.67±8.16	52.51±4.47

Note: % Δ viscosity = (viscosity of 90th days storage of the gel – initial viscosity of the gel)/initial viscosity of the gel

The viscosity of formula abc, bc and ac decreased extensively. Formula bc was gave the highest alteration of gel viscosity (61.15%) while formula a was the lowest one (15.20%) (Table II).

Ca-alginate provided the greatest contribution on the alteration of the viscosity followed by CMC-Na and Carbopol. Formula a showed the lowest alteration, it proving that carbopol gave the smallest contribution among other gelling agents. In contrast to Ca-alginate and CMC-Na which increased the alteration of the gel viscosity, carbopol would reduce the alteration. In other word, carbopol as gelling agent of wound healing gel of ethanolic extract of binahong could maintain the gel viscosity.

Ca-alginate network formation will “collaps” at temperature ranging from 35 to 45°C. This collaps could be caused by a phase separation of the mixture or by over association between alginate and other polymer (Panouillé and Larreta-Garde, 2009). It explained the high contribution of Ca-alginate to alteration of gel viscosity. The collaps network formation of Ca-alginate meant lost or reducing “egg-box” structure of Ca-alginate gel. The reducing “egg-box” structure caused reducing gel viscosity.

The alginate is a linear unbranched copolymer composed of β-D-mannuronate residues and α-L-guluronates residues. The guluronate residues will bind ionically with Ca^{2+} to form metal alginate gel complexes. The metal alginate gel complexes interact ionically with monochain alginate to form alginate gel cluster (Zhao *et al.,* 2011) which is called “egg box” structure. In the degradation proccess, the alginate gel cluster turned into alginate gel complexes then into alginate monochain. The alginate monochain can be degraded into β-D-mannuronate residues and α-L-guluronates residues. These changes will reduce the gel viscosity. It is the reason of high contribution of Ca-alginate in the alteration of gel viscosity.

In aqueous environment, association of macromolecules of CMC-Na occurs through inter-molecular hidogen bonding (Gushchin *et al.,* 2008) and intra-molecular hidrogen bonding (Bochek *et al.,* 2002) of carboxy and hydroxy

Table III. ANOVA and effects value of model of viscosity and bioadhesion properties during storage.

Model	Viscosity		Bioadhesion	
	Prob>F	effects	Prob>F	effects
Carbopol (A)	< 0.0001	-5.91	0.1824	0.43
CMC-Na (B)	< 0.0001	9.54	< 0.0001	9.36
Ca-alginat (C)	< 0.0001	30.73	< 0.0001	3.11
AB	0.0568	-1.79	< 0.0001	5.33
AC	0.2098	-1.17	< 0.0001	9.27
BC	0.0325	2.03	< 0.0001	8.28
ABC	0.8880	0.13	< 0.0001	7.63

Note: Values of "Prob>F" less than 0,0500 indicate that model terms are significant

group of CMC. The breaking of hydrogen bonding caused the reducing the gel viscosity. The degradation of CMC-Na is dominant at high concentration (Liu *et al.,* 2005). The increasing concentration of CMC-Na in the solution increased CMC-Na macromolecules which may be degradated and then increased the alteration of the gel viscosity. The degradation of CMC-Na can be occur either in the cross-link or the polymer backbone.

The interaction between CMC-Na and Ca-alginate statistically significant to the alteration of gel viscosity. CMC-Na and Ca-alginate are gelling agent in the process of gel forming can be able to interact with ionic cation. Na^{+} and Ca^{2+} affected gel formation that was formed by them. In the system, CMC-Na crosslinked with Ca^{2+} through ionic bonding. This association enhance the gel viscosity. The cross-link between CMC-Na and Ca^{2+} from Ca-alginate was stable at low temperature and unstable at high temperature. If cross-link between CMC-Na and Ca-alginate is collapse, it will reduce the gel viscosity. It explained the highest alteration of viscosity of bc formula which contains high concentration of CMC-Na and Ca-alginate.

Carbopol affected the alteration of viscosity through reducing cross-link in the chain. The alteration of viscosity of formula a is smaller than formula 1. It showed that the network formation formed by Carbopol can be able to be stable and support the network formation of the whole system.

The interaction between Carbopol and other gelling agent (CMC-Na and Ca-alginate) will reduce the alteration of gel viscosity. Carbopol interacted with CMC and alginate through hydrogen bonding instead of ionic interaction of CMC-Na and Ca-alginate that have been "collapsed". It explained that the interaction between Carbopol and CMC-Na or Ca-alginate reduced the alteration of gel viscosity (Table III).

Carbopol maintained the viscosity of the system because of its ability to maintain the network formation and to interact with other gelling agent through hydrogen bonding. The increasing of the concentration of carbopol in the gel enhanced its ability to maintain the gel viscosity and reduced the alteration of viscosity of the wound healing gel of ethanolic extract of binahong.

In all formula, bioadhesion of the wound healing gel of ethanolic extract of binahong tends to decrease during storage. Formula abc showed the greatest lowering bioadhesion (60.95%), while formula ac showed the smallest one (30.35%) (Table IV). Statistical analysis showed that the model was valid to predict the value of the bioadhesion alteration of the gel. CMC-Na and Ca-alginate affected the alteration of bioahesion of the gel (Table III).

CMC-Na provided the greatest contribution in the alteration of bioadhesion compared to carbopol and Ca-alginate. It means that the increasing concentration of CMC-Na enhancing the alteration of bioadhesion (lowering the bioadhesion properties of the gel). The degradation reaction of CMC-Na in the system resulted glucose or other degradation product (Bouchard *et al.,* 1989). The degradation product of CMC have a less number of group that can interact with biological tissue compared to that of CMC. It explained that bioadhesion properties of the degradation product is lower than CMC. Ca-alginate provided smaller contribution to the alteration of bioadhesion of the gel than that of CMC-Na.

Carbopol provided the smallest contribution to the alteration of the bioadhesion properties. This contribution was statistically insignificant. It meant that carbopol did not alter the bioadhesion properties of the gel during 3 months storage. In the other word, carbopol can maintain bioadhesion properties of the wound healing gel of ethanolic extract of binahong during storage. The ability of carbopol to maintain bioadhesion properties is related to the ability to maintain the network formation in the system.

CONCLUSION

CMC-Na and Ca-alginate increased the alteration of physical properties of wound healing gel of ethanolic extract of binahong during storage. Carbopol maintained the physical properties of the gel during storage.

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REFERENCES

Bochek AM., Yusupova LD., Zabivalova N.M. and Petropavlovskii GA. 2002. Rheological Properties of Aqueous H-Carboxymethyl Cellulose Solutions with Various Additives. *Rus. J. Appl. Chem.*, 75, 4–7.

Bouchard J., Abatzoglou, N., Chornet E. and Overend RP. 1989. Characterization of depolymerized cellulosic residues. *Wood Sci. Technol.*,**23**, 343–355.

Cockbill S., 2002, Wounds The Healing Process., *Hospital Pharmacist*, Vol 9, Oktober 2002, 255 – 260

Cornell MD., Kaiser NE., and Brenden RA., 1997, Wound Hydrating Gel with Novel Preservative System and Low Cytotoxicity, *United States Patent,* Patent number 5670169

Gushchin Na., Ostrovidova GU., and Sosnov E a., 2008. Antimicrobial polymeric composite films for medical use. *Rus. J. Appl. Chem.*, 81, 132–135.

Kumar L., and Verma R., 2010. In vitro evaluation of topical gel prepared using natural polymer. *Int. J. Drug Deliv.*, 2, 58–63.

Lim SW., Hong SP., Jeong SW., Kim B., Bak H., Ryoo HC., Lee SH. and Ahn SK. 2007. Simultaneous effect of ursolic acid and oleanolic acid on epidermal permeability barrier function and epidermal keratinocyte differentiation via peroxisome proliferator-activated receptor-alpha. *J.Dermatol.*, 34, 625–34.

Lin H-Y., Kuo S-C., Chao P-DL., and Lin T-D., 1988. A new sapogenin from boussingaultia gracilis. *J. Nat. Prod.*, 5, 797–798.

Liu P., Peng J., Li J., and Wu J., 2005. Radiation crosslinking of CMC-Na at low dose and its application as substitute for hydrogel. *Radiat. Phys. Chem.*, 72, 635–638.

Mallefet P., and Dweck, A.C., 2008, Mechanism of Wound Healing Examined, *Personal Care*, 75 – 83

Martens P., Metters AT., Anseth KS. and Bowman CN., 2001. A Generalized Bulk-Degradation Model for Hydrogel Networks Formed from Multivinyl Cross-linking Molecules. *J.Phys. Chem.*, 105, 5131–5138.

Meyvis TKL., De Smedt SC., Demeester J. and Hennink WE. 2000. Influence of the Degradation Mechanism of Hydrogels on Their Elastic and Swelling Properties during Degradation. *Macromolecules.,* 33, 4717–4725.

Panouillé M. and Larreta-Garde V. 2009. Gelation behaviour of gelatin and alginate mixtures. *Food Hydrocolloids.*, 23, 1074–1080.

Wittaya-areekul S., Prahsarn, C., and Sungthongjeen S. 2006. Development and in vitro evaluation of Chitosan-Eudragit RS 30D composite wound dressings. *AAPS PharmSciTech.*,7, E30.

Zhao Y., Hu F., Evans JJ. and Harris MT. 2011. Study of sol–gel transition in calcium alginate system by population balance model.*Chem.Eng.Sci.*, 66, 848–858.

Zatz JL., and Kushla GP., 1996., Gels, in Lieberman HA., Rieger MM., and Banker GS., *Pharmaceutical Dosage Forms: Disperse System Vol 2.* ed 2, Marcell Dekker Inc., New York