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Hydrogel Formulation of Usnic Acid and Antibacterial Activity Test Against *Propionibacterium acne*

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Abstract

Usnic acid is known for its remarkable antimicrobial activity. The aim of this research was to formulate hydrogel of usnic acid and evaluate the antibacterial activity against Propionibacterium *acne*. In this study, isolated usnic acid was formulated in hydrogel dosage form using several gelling agents: Aqupec HV-505, sodium alginate and HPMC K 100M. Due to the low solubility of usnic acid, solid dispersion of usnic acid was prepared using PVP K-30 at ratio 1:2 (w/w) by freeze drying techniques. Prior to preparation of hydrogel, concentration of gelling agent was optimized, which were 0.15, 0.175, 0.2% of Aqupec HV-505; 1.5, 1.75, 2% of sodium alginate, and 1.5, 1.75, 2% of HPMC K 100M. All of the gelling agents showed homogenous gel, pH at range 5.37 – 6.33 and viscosity in range 259.07 – 10,759.00 cps. Hydrogel was prepared by dispersing 1% intact usnic acid and 3% freeze dried usnic acid in three different gelling agents. The hydrogel of usnic acid was evaluated for pH, viscosity, stability test and microbiology test. The amount of usnic acid in hydrogel was determined by spectrophotometry UV-Vis. Hydrogel with intact usnic acid showed non-homogenous gel, while hydrogel usnic acid in solid dispersion was homogenous. pH of hydrogel was in range 5.5 – 6.4 and viscosity was 2017.03 – 3866.52 cps. The result of syneresis test was in a range 10.94 – 11.86 g for 72 hours. All the hydrogel was stable after two months' storage at room temperature. The diameter inhibition of hydrogel was in a range 20 – 32 mm. The amount of usnic acid in hydrogel was in range 95.9 – 99.23%. In conclusion, usnic acid in solid dispersion is able to formulate in hydrogel better than intact usnic acid.

Keywords: usnic acid, solid dispersion, hydrogel, solid dispersion, *Propionibacterium acne*

Introduction

Unic acid, a secondary metabolite produced by Usnea sp, is already known for many pharmacological activities including anti-bacteria (Madamombe & Afolayan, 2003), antivirus (Perry, et al., 1999), anti-proliferation (Campanella, et al., 2002), anticancer (Mayer, et al., 2005), antioxidant (Behera,et al., 2005), antipyretic and analgesic (Okuyama, et al., 1995) and anti-inflammation (Vijayakumar, et al., 2000). Usic acid also has a significant activity against anaerobic Gram positive bacteria including Propionibacterium species (Ingolfsdottir, 2002). *Propionibacterium acne* is one of the normal flora on the skin that in certain conditions can cause inflammation by producing lipases which break down fatty acids free of skin lipids, known as acnes (Khan, et al., 2009). This bacterium is included as facultative anaerobic bacteria and classified as Gram positive (Khan, et al., 2009).

The numerous activities of usnic acid, yet is still limited available in market for pharmaceutical products. In Indonesia, there is only one commercial product that has been marketed, Cream Scabicid®. This limited number of product is likely due to low solubility of usnic acid in water (0.01 g/100 ml) (Cocchietto, et al., 2002). Some efforts have been done to increase the

solubility of usnic acid, such as complex formation using cyclodextrin, microencapsulation using polymer PLGA, milling process (Zaini, OJC), and preparation in solid dispersion using HPMC (lili, OJC). Our recent study has shown enhancement of usnic acid by preparing in solid dispersion using poly-vynil-pirolidon (PVP) K-30 and the potency as anti-oxidant was in accordance with the solubility result (lili, rasayan).

The only application of usnic acid as antibacterial or anti acne has been investigated by converting into salt form or metal salt (Eady et al., 2013). Based on the above considerations, preparation of hydrogel containing intact usic acid and usnic acid-solid dispersion is done in this research. Solid dispersion was prepared using PVP K-30 in order to increase the solubility of usnic acid. Prior to preparation of usnic acid hydrogel, concentration of gelling agents used is optimized. Hydrogel of usnic acid was then evaluated by homogeneity, pH, stability, and anti-bacterial test against *Propionibacterium acne*.

Materials and methods

Materials

Usnic acid (isolated from Usnea sp as explained in previous work, Zaini ojc milling), PVP K-30 (Shin-Etsu Chemical, Japan), aqupec 505 HV (Sumitomo Seika Chemicals Co., Ltd, Japan), sodium alginate (PT. Kimia Farma, Indonesia), HPMC K100 M (PT. Kimia Farma, Indonesia), glycerin (Bratachem, Indonesia), triethanolamine (Bratachem, Indonesia), nutrient agar (Merck, Germany), Propionibacterium acnes ATCC 6919 (2The Laboratory of Natural Resource of Sumatra, Indonesia), clindamycin phosphate gel (Medi-Klin, PT. Surya Dermato Medika Indonesia), phosphate buffer (Merck, Germany), chloroform (Merck, Germany), ethanol (Bratachem, Indonesia), and distilled water.

Methods

Optimization of gelling agent for hydrogel base

Formulation of hydrogel was prepared using three different gelling agents which are Aqupec 505 HV, Sodium alginate and HPMC K-100 at different concentration. Hydrogel was prepared by dispersing the gelling agent in distilled water until its swelling well.

Table 1. Preparation of hydrogel base

Materials	Formula 1			Formula 2			Formula 3		
	1a	1b	1c	2a	2b	2c	3a	3b	3c
Aqupec 505 HV (%)	0.15	0.175	0.20	-	-	-	-	-	-
Sodium alginate (%)	-	-	-	1.5	1.75	2	-	-	-
HPMC K100 M (%)	-	-	-	-	-	-	1.5	1.75	2
Glycerin (%)	10	10	10	10	10	10	10	10	10
TEA (%)	0.4	0.4	0.4	-	-	-	-	-	-
Distilled water ad (%)	100	100	100	100	100	100	100	100	100

Evaluation of hydrogel base

a. Organoleptic test
   Each of hydrogel was then evaluated by its appearance visually.
b. Homogeneity
   About 1 g of each hydrogel sample was dispersed on an object glass and observed the homogeneity
c. pH

Each of hydrogel was diluted into 1% concentration and pH of each hydrogel was determined using pH meter digital (Hanna Instruments, the USA).

d. Viscosity test
   Viscosity test of each hydrogel was conducted using a Brookfield viscometer (xx, yy).
e. Wash out test
   The wash out test was carried out by applying 1 g each hydrogel to the hand then washed by water. The amount of water to wash out the hydrogel is calculated.

Preparation solid dispersion

Solid dispersion of usnic acid PVP K-30 was prepared as described in previous work (lili, rasayan).

Preparation of Hydrogel Usnic acid (UA) and Usnic Acid in Solid Dispersion (UA-SD)

The optimum concentration of each gelling agent was prepared for hydrogel containing intact usnic acid and usnic acid in solid dispersion. The amount of usnic acid dispersed in hydrogel was equivalent to 1% (w/w), so that the amount of usnic acid-solid dispersion was 3% (w/w). Each of optimal concentration of gelling agent was used in this hydrogel preparation.

Table 2. Hydrogel formula of intact usnic acid and solid dispersion

Materials	Formula 1 (%)		Formula 2 (%)		Formula 3 (%)	
	UA	SD	UA	SD	UA	SD
Solid dispersion	-	3%	-	3%	-	3%
Intact usnic acid	1%	-	1%	-	1%	-
Hydrogel base	99 %	97%	99 %	97%	99 %	97%

Evaluation of Hydrogel Usnic acid (UA) and Usnic Acid in Solid Dispersion (UA-SD)

a. Six hydrogel preparations were evaluated for organoleptic test, homogeneity, pH and viscosity as described in evaluation of hydrogel base.
b. Usnic acid assay
   The amount of usnic acid in hydrogel was determined by a spectrophotometer UV-Vis (Shimadzu, Japan) at maximum absorption length in phosphate buffer. About 1 g of each hydrogel was dissolved with 100 ml phosphate buffer pH 7.4 and homogenized by sonication for 2 hours. The sample was then filtered and measured the absorbance at 217 nm. The amount of usnic acid was then calculated based on linear regression.
c. Spreadability test
   About 0.5 g of hydrogel was placed on transparent glass equipped with on a graph paper. The hydrogel was covered with transparent plastic and given a certain load (1, 3, 5, and 7 g) for 15 seconds. The diameter was measured after being given a load.
d. Syneresis test
   Syneresis test was done by storing the hydrogel at 10°C for 24, 48 and 72 hours. Each hydrogel was placed on a cup to hold water released from the gel during storage. Syneresis value was calculated by measuring the weight loss during storage then compared to the initial weight.
e. Cycling test

The hydrogel was tested for stability against cooling condition. Each sample was stored at temperature of 0 - 4°C for 24 hour. The homogeneity of each sample was observed and pH was determined after the cycling test.

f. Stability test
Each sample of hydrogel was kept in at room temperature for 8 weeks. The stability of sample was observed every week including the physical appearance, homogeneity, and pH test.

g. Antibacterial activity **[25][26]**
One ml of *Propionibacterium acne* suspension stock was put into a sterilized petri dish and added nutrient agar medium. The mixture was homogenized. About 10 mg of each hydrogel sample was then put on the wells that has been prepared and incubated for 24 hours at 37 °C. The ability of hydrogel samples to inhibit the growth of *Propionibacterium acne* was determined by measuring diameter inhibition. As positive and negative controls, a marketed gel containing clindamycin phosphate 1.2% (Medi-klin) and hydrogel base were tested by the same procedure.

Result and Discussion

Propionibacterium acne, a Gram-positive and facultative anaerobic bacterium, is normally found on skin in normal condition. However, when the skin produces more sebum which causes pilosebaceous unit increases, this bacterium tends to multiply and induced infection process that namely acne vulgaris. Several studies have shown that some metabolites from plants have antibacterial activity against *P. acnes*, including usnic acid from *Usnea barbata* that has ability to inhibit the growth of bacterium at concentration ≥ 1µg/mL (68) (Hamid Nasri, 2015). One of the most favorable preparations used in handling acne is hydrogel. Hydrogel offers convenience in used and has more attractive appearance due to its transparency compared to other preparations. Moreover, hydrogel is easily washed out and give cool sensation during the gel application on skin due to water content of the base. Therefore, gelling agent plays important part in preparing hydrogel. Chitosan, HPMC, Carbomer, HPMC, PVA and sodium alginate are commonly used as gelling agents.

The result of hydrogel base optimization is shown in Table 3. Based on the result, all the hydrogel base was homogenous with pH in a range 5.4 – 6.4 which is acceptable in preparing hydrogel. However, the viscosity of the F1a was the lowest which was relatively as liquid than gel. The wash out result denoted that the higher concentration of gelling agent, the more water need to wash the gel out of the skin. Thus, preparation of hydrogel containing intact usnic acid (UA) and usnic acid-solid dispersion (UA-SD) was carried out using F1b, F2a and F3a.

Table 3. Result of hydrogel base optimization

No	Hydrogel base	Homogeneity	pH	Volume of wash out	Viscosity
1	F1a	Homogenous	6.33	16 ml	259.07 cps
2	F1b	Homogenous	6.20	17 ml	2595.39 cps
3	F1c	Homogenous	6.53	18 ml	3703.75 cps
4	F2a	Homogenous	5.40	32 ml	3815.19cps
5	F2b	Homogenous	5.37	37 ml	5115.17 cps
6	F2c	Homogenous	6.00	40 ml	14203.22 cps
7	F3a	Homogenous	5.80	40 ml	5544.87 cps
8	F3b	Homogenous	5.40	50 ml	9811.03 cps
9	F3c	Homogenous	5.40	60 ml	15811.03 cps

The result of homogeneity, pH, volume of wash out, viscosity, and usnic acid content in hydrogel usnic acid and usnic acid-solid dispersion is shown in Table 4. The hydrogel of intact usnic acid (UA) was not homogenous while usnic acid-solid dispersion (UA-SD) was homogenous. This result has been predicted since the intact usnic acid crystal could not be well dispersed in hydrogel base. Meanwhile, the addition of usnic acid in hydrogel influence the pH result. Usnic acid is a weak acid that has pKa 4.4 (Margret Bessadottir, 2012). Moreover, PVP K-30, used to enhance the solubility of usnic acid, reduced the viscosity of hydrogel. This phenomenon was also observed in other study (Katarzyna Małolepsza-Jarmołowska, 2010), in which PVP maintains adhesion and limits of the physiological range. The amount of usnic acid in hydrogel was almost close to each formula which indicated that all intact usnic acid and usnic acid-solid dispersion has been the same amount in hydrogel preparation.

Table 4. Result of hydrogel test

Formula	**Homogeneity**	**pH**	**Viscosity**	**Usnic acid assay**
F1b UA	No	5.63 ± 0.09	2328.67 cps	97.56 ± 0.19
F1b UA-SD	Homogeneous	5.67 ± 0.05	2270.74 cps	99.23 ± 0.29
F2a UA	No	5.70 ± 0.08	2038.95 cps	96.94 ± 0.51
F2a UA-SD	Homogeneous	5.50 ± 0.08	2017.03 cps	98.19 ± 0.10
F3a UA	No	5.67 ± 0.05	3612.63 cps	96.90 ± 0.29
F3a UA-SD	Homogeneous	5.50 ± 0.14	3635.04 cps	97.77 ± 0.29

Spread-ability and syneresis test of hydrogel UA and UA-SD were also conducted to characterize the hydrogel as seen in Table 5. The spread-ability value was corresponding to the viscosity result in previous evaluation. According to the result, the lower viscosity of hydrogel the greater ability to spread over. Meanwhile, syneresis is the ability of gel to be shrinkaged due to change in temperature. The synersis result of all hydrogel were almost similar.

Table 5. Spread-ability and syneresis test result

Formula	**Spreadability (cm)**					**Syneresis (g)**		
	Load 0 g	**Load 1 g**	**Load 3 g**	**Load 5 g**	**Load 7 g**	**24 h**	**48 h**	**72 h**
F1b UA	1.90	2.30	2.75	3.25	3.90	11.20	11.13	11.11
F1b UA-SD	2.85	3.45	3.95	4.45	5.10	11.18	11.15	11.14
F2a UA	1.85	2.35	2.70	2.85	3.00	11.05	11.37	11.31
F2a UA-SD	2.05	2.45	2.70	2.95	3.10	11.03	11.03	10.93
F3a UA	2.20	2.70	3.00	3.20	3.40	11.02	11.95	10.95
F3a UA-SD	2.40	2.85	3.15	3.55	3.65	11.04	10.94	11.01

The result of cycling test in Table 6 indicated the stability of hydrogel under cool temperature, while stability of hydrogel at room temperature for eight weeks can be seen in Table 7. The cycling test did show similar result of two parameters before and after the test. Similarly, the stability test was relatively the same after eight-week storage which suggested all hydrogel was stable.

Table 6. Cycling test result

Formula	**pH**		**Homogeneity**	
	Before	**After**	**Before**	**After**

F1b UA	5.63 ± 0.09	5.67 ± 0.00	No	No
F1b UA-SD	5.67 ± 0.05	5.67 ± 0.03	Homogeneous	Homogeneous
F2a UA	5.70 ± 0.08	5.63 ± 0.05	No	No
F2a UA-SD	5.50 ± 0.08	5.47 ± 0.05	Homogeneous	Homogeneous
F3a UA	5.67 ± 0.05	5.63 ± 0.05	No	No
F3a UA-SD	5.50 ± 0.14	5.20 ± 0.02	Homogeneous	Homogeneous

Table 7. Stability test at room temperature

Formula	**pH Homogeneity**			
	Week 1	**Week 2**	**Week 4**	**Week 8**
F1b	6.30 Homogeneous	6.20 Homogeneous	6.20 Homogeneous	6.20 Homogeneous
F1b UA	5.63 No	5.81 No	5.83 No	5.83 No
F1b UA-SD	5.67 Homogeneous	5.63 Homogeneous	5.63 Homogeneous	5.63 Homogeneous
F2a	6.60 Homogeneous	6.30 Homogeneous	6.00 Homogeneous	6.00 Homogeneous
F2a UA	5.50 No	5.50 No	5.50 No	5.47 No
F2a UA-SD	5.70 Homogeneous	5.70 Homogeneous	5.70 Homogeneous	5.70 Homogeneous
F3a	5.83 Homogeneous	5.73 Homogeneous	5.73 Homogeneous	5.73 Homogeneous
F3a UA	5.50 No	5.43 No	5.43 No	5.43 No
F3a UA-SD	5.57 Homogeneous	5.57 Homogeneous	5.57 Homogeneous	5.57 Homogeneous

The result of previous evaluations was anticipated to the antibacterial activity of usnic acid in hydrogel preparation. The diameter zone inhibition of each sample was correlated to the activity of antibacterial assay against *P. acne*, which is shown in Table 8 and Figure 1.

Table 8. Result of anti-bacterial assay

Formula	**Diameter inhibition (mm) ± SD**
F1b	0 ± 0.00
F1b UA	30 ± 0.05
F1b UA-SD	32 ± 0.05
F2a	0 ± 0.00
F2a UA	29 ± 0.05
F2a UA-SD	30 ± 0.05
F3a	0 ± 0.00
F3a UA	20 ± 0.05
F3a UA-SD	26 ± 0.05
Positive control (+)	30 ± 0.05

All the hydrogel base or negative control showed no inhibition zone which indicated that no activity of antibacterial activity, see Fig 1a. Meanwhile, the positive control which used clindamycin gel shows about 30 mm diameter zone inhibition, see Fig 1b. The largest diameter inhibition was the hydrogel contained usnic acid-solid dispersion using Aqupec as the gelling agent. This result pointed that the hydrogel of usnic acid which prepared with different gelling agents had the same efficacy in treating acne compared to the marketed and synthetic antibiotics. The potency of usnic acid as antibacterial is owing to the phenolic groups that known to have antibacterial activity (Hamid Nasri, 2015).

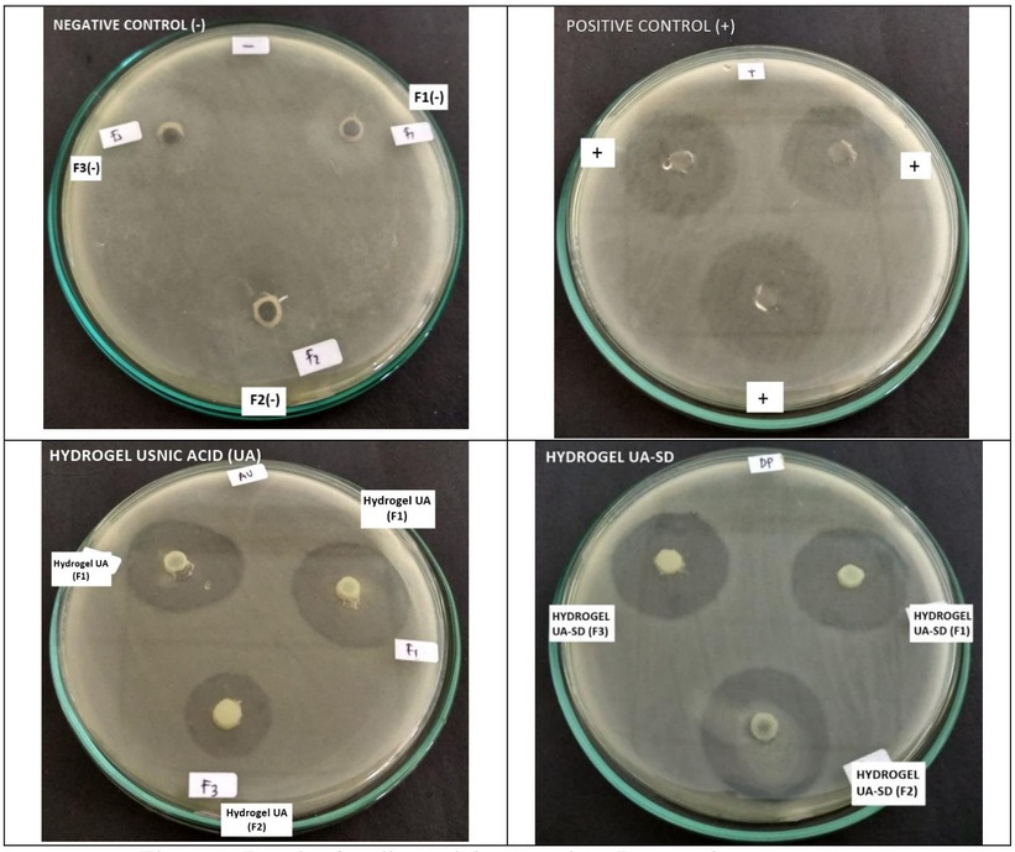

Figure 1. Result of antibacterial test against *Propionibacterium acne*

Conclusion

Preparation of hydrogel is influenced by the form of usnic acid used, which solid dispersion of usnic acid with PVP K-30 provides better result in appearance compared to intact usnic acid. The antibacterial activity of hydrogel usnic acid and usnic acid-solid dispersion was almost similar to the marketed gel contained synthetic antibiotic.

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References

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