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The Accelerated Stability Test for Snakehead Fish Ointment and Kelulut Honey Using BHA as an Antioxidant

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Abstract
The ointment of snakehead fish extract and kelulut honey has a potential to boost the wound recuperation process. It is formulated for topical application using adeps lanae as the base, which is prone to oxidation, leading to instability. The addition of antioxidants is one way to prevent the oxidation process in the preparation. This has a look at aims to decide the impact of the addition of BHA as an antioxidant in the ointments of snakehead fish extract (*Channa striata*) and kelulut honey (*Heterotrigona itama*) at 28 days of storage with an accelerated stability test method with a temperature 40 ± 20oC and RH 75±5%. The ointment was made with various concentrations of BHA 0.005% (F1), 0.01% (F2), and 0.02% (F3) then tested the physical properties of the ointment and analyzed by SPSS. Results showed that spreadability increased significantly in F1, F2, and F3 compared to F0. Adhesion tests indicated reduced bonding strength with significant differences among formulas. The acid number decreased in F2 and F3, suggesting better oxidation prevention. Organoleptic, homogeneity, and protective power tests confirmed all formulas remained stable. Among them, F2 was the most effective in preventing oxidation, as indicated by the lowest acid number.

Keywords: Accelerated Stability, BHA, Kelulut honey, Snakehead Fish.

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Uji Stabilitas Dipercepat Sediaan Salep Ekstrak Ikan Gabus dan Madu Kelulut menggunakan BHA sebagai Antioksidan

Abstrak
Salep yang mengandung ekstrak ikan gabus dan madu kelulut memiliki potensi untuk mempercepat proses pemulihan luka. Salep ini diformulasikan untuk aplikasi topikal dengan menggunakan adeps lanae sebagai basis, yang rentan terhadap oksidasi sehingga menyebabkan ketidakstabilan. Penambahan antioksidan merupakan salah satu cara untuk mencegah proses oksidasi dalam sediaan. Penelitian ini bertujuan untuk menentukan pengaruh penambahan BHA sebagai antioksidan dalam salep ekstrak ikan gabus (*Channa striata*) dan madu kelulut (*Heterotrigona itama*) selama penyimpanan 28 hari menggunakan metode uji stabilitas dipercepat pada suhu 40 ± 2°C dan kelembaban relatif (RH) 75 ± 5%. Salep dibuat dengan berbagai konsentrasi BHA, yaitu 0,005% (F1), 0,01% (F2), dan 0,02% (F3), kemudian diuji sifat fisiknya dan dianalisis menggunakan SPSS. Hasil penelitian menunjukkan bahwa daya sebar meningkat secara signifikan pada F1, F2, dan F3 dibandingkan dengan F0. Uji adhesi menunjukkan penurunan kekuatan ikatan dengan perbedaan yang signifikan antar formula. Angka asam menurun pada F2 dan F3, menunjukkan pencegahan oksidasi yang lebih baik. Uji organoleptik, homogenitas, dan daya lindung mengonfirmasi bahwa semua formula tetap stabil. Di antara semua formula, F2 paling efektif dalam mencegah oksidasi, sebagaimana ditunjukkan oleh angka asam terendah.

Kata Kunci: BHA, Ikan gabus, Madu kelulut, Stabilitas dipercepat.

1. Introduction

Ointments are semi-solid preparations meant for topical use and are easy to apply. The requirement for giving the ointment is that the ointment must not have a rancid smell. The selection of an ointment base must be stable, and suitable for the active substance to be used in the preparation.[1] Snakehead fish extract has various properties including accelerating wound healing.[2] Honey has some essential characteristics such as anti-inflammatory, antibacterial, and antioxidant activity, the ability to stimulate the process of removing dead tissue or debridement, decreasing smell in wounds, and keeping wound moisture which in turn can help boost up wound recuperation.[3]

This ointment formulation used an adeps lanae as a basis. Base adeps lanae or fleece fat is a purified fat-like substance.[4] The oil phase is more susceptible to oxidation because it has a double bond of a polynomial (unsaturated fatty acids). The double bond will drop out and bind with oxygen from the air to cause rancidity. The addition of antioxidant compounds can slow down the oxidation of the material.[5] *Butylated hydroxyanisole* is a synthetic antioxidant that is known to have higher antioxidant activity than vitamin E. In the BHA structure, there is a conjugated aromatic ring which is the active part of BHA. This aromatic ring can act as a stabilizer for free radicals.[6] BHA used as an antioxidant in the topical preparation is in the range of 0.005% - 0.02%.[7] The researchers are interested in adding excipients that can prevent the occurrence of oxidation reactions in ointment preparations of a combination of snakehead fish extract (*Channa striata*) and kelulut honey (*Heterotrigona itama*) such as BHA.

Examination of the stability of the drug is necessary to ensure the quality of the preparation.[8] Accelerated stability test performed at 40° ± 2°C / 75% RH ± 5% for 28 days, then were observed organoleptic, adhesiveness, dispersive power, homogeneity, and the acid number. Assessments were carried out on days 1, 3, 7, 14, 21, and 28 to observe the physical stability of snakehead fish and kelulut honey ointment with the addition of BHA as an antioxidant.

2. Materials and Methods

2.1. Materials

The materials used in this observe included snakehead fish extract (*Channa striata*), kelulut honey (*Heterotrigona itama*), adeps lanae (CV. Clorogreen), CMC-Na (CV. Clorogreen), propylparaben (PT. Brataco), methylparaben (PT. Brataco), propylene glycol (Green Pharmacy), and BHA (CV. Clorogreen).

2.2. Methods

2.2.1. Preparation of snakehead fish extract

Approximately 1 kg of snakehead fish has cleaned the top and the belly of the fish. Then it was in a pan for a 30 minutes at a temperature around of 60°C to 70°C. The meat of the snakehead fish is wrapped in a cotton fabric and put in hydraulic press equipment. Then pressed using high pressure to get the extract. Then the extract will be sentrifuge till get the white liquid extract. The obtained snakehead fish extract shouldn't be kept at room temperature for more than 12 hours. This is done to prevent damage to the protein content found in the aqueous phase of the snakehead fish extract and to maintain the extract quality. The extract obtained is inserted into a test tube and wrapped using a clean pack first and then layered with aluminum foil.[9]

2.2.2. Preparation of ointment

Ointment formulation can be seen in Table 1. Each ingredient is required for the ointments to be prepared and weighed. Then the extract and kelulut honey was heated at 50°C after that put into a mortar. Then added CMC-Na and stirring until homogeneous. The adeps lanae was melted in a vaporizer at 70°C and put it in a different mortar. Crushed adeps until yellowish-white, then added BHA ground until homogeneous (second mixture). Put the first mixture into the mortar containing a second mixture, and crushed until homogeneous. After a homogeneous preparation was added methylparaben and propylparaben were previously dissolved with propylene glycol. The preparation was

Table 1. Ointments Formulation (%w/v)

Ingredients	F0 (%)	F1 (%)	F2 (%)	F3 (%)
Snakehead Fish Extract	30	30	30	30
Kelulut Honey	30	30	30	30
BHA	-	0,005	0,01	0,02
CMC-Na	3	3	3	3
Methyl Paraben	0,18	0,18	0,18	0,18
Prophyl Paraben	0,02	0,02	0,02	0,02
Propilenglycol	qs	qs	qs	qs
Adeps Lanae	Ad 100	Ad 100	Ad 100	Ad 100

ground until homogeneous and then the preparation was put into the ointment pot. The ointment was made in three replications. Then put the prepared ointment into the stability cabinet at a temperature of 40±20°C and an RH of 75±5%.

2.2.3. Evaluation of the Preparation Stability

Organoleptic Test

The organoleptic test includes texture, color, and odor observation. It was carried out before and after accelerated conditions, the smell is not rancid and the textured soft but not break.[10] It was observed visually during storage for 28 days.

Homogeneity Test

A homogeneity test was carried out by using a glass object. A weight of 0.1g ointment smeared on a slide and covered then observed homogeneity of the sample. The preparation was said to be homogeneous if not coarse at the object-glass, there are no clumps.

Protection Test

The test of the protection power of the ointment was carried out using 0.1 N KOH and phenolphthalein compounds. Wetted filter paper (10 x 10 cm) with phenolphthalein and dried. Weighed 1 gram of the preparation then smeared it on the filter-out paper. On filter-out paper made place (2.5 x2.5 cm) and made dike with solid paraffin melted at the edge. Put the filter-out paper (2.5 x 2.5 cm) on top of the previous filter-out paper. Then a 0.1 N KOH solution was dropped at the area, observed for 5 minutes. If no stains appear on the filter paper, it means that the preparation can provide protection.[11]

Adhesion Test

The adhesion test was carried out by weighing 0.25 grams of ointment on the object-glass and put another slide on top of the sample. After adding the weight of 1 kg for about 5 min on the object glass and mounted on the test equipment. 80g heavy load is released, noting the time until the second glass object is apart. It was observed during storage for 28 days.[12]

Spreadibility Test

1g ointment was weighed and located within the middle of the glass. Then put a glass lid over the sample and be allowed to stand for 1 minute. Added extra weight of 50 grams. Record the diameter until an additional load of 150 grams. The diameter of a good spread of semi-solid preparation is between 5-7 cm.[13] Observations were made during a storage duration of 28 days on test points on days 1, 3, 7, 14, 21, and 28.

Acid Number Test

Weighed as much as 10 grams of the preparation and then put into an Erlenmeyer, added 50 mL of 90% alcohol, then heated for 10 minutes on a water bath while stirring. Then titrated the solution with 0.0986 N KOH using a phenolphthalein indicator until it forms a light pink color.[14]

Data Analysis

The results of the organoleptic take a look at, homogeneity test, and protection power test will be presented in a table. The results of adhesive and spreadability test for each formula will test statistically Oneway ANOVA using the SPSS Statistics 21.0 program with a confidence level (α 0.05).

3. Results

3.1. Organoleptic Test

The results of organoleptic test has been shown in table 2. All of the preparations did not change in shape or consistency. The consistency and texture of the preparation is soft but not broken. This could be due to the combination of snakehead fish extract, kelulut honey and adeps lanae are able to absorb water. The color of all preparations tends to be the same as yellowish white. It was caused by the concentration of the base and the active substance used is large enough so that it can affect the color of the preparation. The smell of this ointment preparation was observed and the results tend the preparation has a distinctive odor of kelulut honey.

Table 2. The average results of organoleptic and homogeneity test

Formulation	Consistency	Color	Odor
F0 (Control)	++	Yellowish-white	Honey smell
F1	++	Yellowish-white	Honey smell
F2	++	Yellowish-white	Honey smell
F3	++	Yellowish-white	Honey smell

Description : Consistency + = brown or oily; ++ = soft; +++ = rough
F0= BHA (0%); F1= BHA (0.005%); F2= BHA (0.015%); F3= BHA (0.02%)

3.2. Homogeneity Test

The homogeneity test results has been shown in table 2. Homogeneity testing is performed to look at the uniform distribution of the active substance in the preparation. This test was observed by visual to look at the uniform distribution during 28 days in a stability cabinet. Based on the results all formulas were homogeneous for 28 days.

3.3. Protection Power Test

The results of protection power test has been shown in table 3. The protection test outcomes show that the combination of snakehead fish extract and kelulut honey ointment can provide protection. Because the test results that there is no pink spot on the filter-out paper after being dripped with 0.1 N KOH.

3.4. Adhesion Test

The adhesion test was observed the adhesion of the ointment at extreme temperatures of 40ºC ±2 ºC and 75±5% RH for 28 days. Adhesion test ointment has shown in Table 4.

The One Way ANOVA analysis results were then tested with the Post Hoc Test. The graphic of the results has shown in figure 1. The adhesive power of each formula on days 1. 3. 7. 14. 21. and 28 was significantly different. Data adhesiveness for each formula will be compared on day 28. A comparison was made on the 28th day of data because it was considered that the preparation had changed. Based the ANOVA analysis test was showed a significant difference among the formulas (F0, F1, F2, F3). Based on Post Hoc Test results the adhesion test between F1. F2. and F3 is not significantly different. However. there are significant differences between F2 to F0 (control). The decrease in the adhesive power of the preparation after 28 days that means BHA can affected the stability of the preparation.

3.5. Spreadability Test

The results obtained show the scattered of all formulas from day 1 to day 28 of the spread tends to increase. The test results of the spreadability has been shown in Table 5. The analysis show it was a significant difference among the formulas (F0, F1, F2, F3) at day 28. The Post Hoc Test results concluded that the test of the spread between F1, F2, and F3 was significantly different on F0. This result shows that the addition of BHA can affect the stability indicated by increasing the dispersion of the preparation.

3.6. Acid Number Test

Tests performed acid number for 28 day at the testing point day 1 and day 28. This test aims to see the difference in acid value. between the first day of testing and the final day of testing. The results test of acid number has been shown in Table 6.

The test results showed an acid value based on damage or rancidity adeps lanae on day 28. In the graph. the volume of KOH used to neutralize the acid formed due to adeps lanae oxidation increased on day 28. Based on calculations obtained an acid number of the smallest acid number is formula 2 with a concentration of 0.01% BHA is 7.622. These results indicate that the BHA 0.01% is the optimal concentration to prevent and inhibit the oxidation processes in the ointment base. The highest acid number value was at F0 as the control without antioxidants. 10.428. Based on these data we can conclude that the addition of BHA affects the stability of ointment preparation by a decrease in acid number.

4. Discussion

The consistency and texture of the ointment are soft but do not break. It could be due to the combination of kelulut honey, snakehead fish extract and adeps lanae as a base can absorb water. There was a CMC-Na that can absorb moisture from the air into the system, and its water content rises, and the system's consistency in the high temperatures and humidity. The coloration of all preparations tends to be equal to yellowish-white. The colour of the preparation caused by the adeps lanae as base and the active substances honey and snakehead fish extract used. The three materials are large enough so that their concentration can affect the color of the preparation. The terms of the ointment can be said to be homogeneous if there are no coarse grains at when the preparation was be applicated or on the slide. This homogeneity testing can also be seen from the spread of color and the presence or absence

Table 3. The average results of Protection power test

Formulation	Spotting color
F0	-
F1	-
F2	-
F3	-

Table 4. The results of adhesion test (n=3, mean ± SD)

Day	F0	F1	F2	F3
1	166.67±21.39	240.67±30.75	207.33±25.89	271.33±42.00
3	147±11.53	204.67±54.20	167±32.51	228.33±16.80
7	125.67±11.24	156.67±28.36	155.33±26.31	195.33±6.15
14	116±7.21	144.33±34.79	135.33±21.36	164.67±17.16
21	105.33±10.07	121±21.63	83.33±5.13	128±22.07
28	88±3.61	79±10.54	52.33±6.66	78.33±18.61

of phase separation on preparations.[15] All formulas showed the results were uniform and homogeneous.

The adhesion test means determining the capability of the ointment preparation to adhere to the skin. The capability of these adhesive power and can influence the therapeutic effect. The longer the ointment preparation was able to stick on the skin. the drug contact with the skin will be durable. and given therapeutic effect is also relatively longer.[16] The parameter on this adhesive test is the release time of the object-glass. The longer it takes to remove the slide. the higher the ability of the ointment to stick to the pores and skin. Based on the adhesion data obtained there was a decrease in the adhesion time for all formulas. This occurrence may be because of external factors such as temperature and humidity inside the stability cabinet. The temperature and humidity could make the preparation absorbs more water in to the system and influence the consistency of the preparation.

A protection power means observing the ability of ointment preparations to protect the skin from external influences such as acids or bases. dust. pollutants. and sunlight. This test uses a strong base compound of 0.1 N KOH. KOH 0.1 N will react with phenolphthalein as an indicator to form a light pink color. The ointment preparation can protect from external influences if there are no stains on the filter-out paper with 0.1 N KOH dripping. The protection test outcomes show that the combination of snakehead fish extract and kelulut honey ointment can provide protection. Because the test results that there is no pink stain after being dripped with 0.1 N KOH on the filter-out paper.

The increase of the spread could be stricken by environmental factors which in the temperature and humidity that used are ludes inside the cabinet's stability. The addition of CMC-Na in the formulas also can influence the spreadability of the preparation. CMC-Na can absorb moisture from the air into the system.[17] Based on the One way ANOVA test it was a significant difference among the formulas (F0. F1. F2. F3) at day 28. The Post Hoc test results concluded that the test of the spread was significantly different between F1. F2. and F3 on F0. The result shows addition of BHA as an antioxidant can increasing the dispersion of the preparation and affect the stability.

Acid number testing is one of the methods to measure the free fatty acids formed in a preparation.[18] The oxidation process can cause rancidity, accompanied by ketones and aldehydes, which were acidic. The principle is the acid number by calculating of KOH that used to neutralize the fatty acid in 1 gram of oil.[19] The hydroxide ions derived from KOH as titrants will be neutral with hydrogen ions derived from acidic compounds in the ointment preparation.[20] The test results showed an acid value based on damage or rancidity adeps lanae on day 28. This damage could be cause by the extreme temperature, oxygen. light. metals. acids. bases. and enzymes.[21] The increase in the number of free fatty acids can also be caused by the presence of water content. It can accelerate the hydrolysis process that can cause rancidity in the preparation. The damage of adeps lanae can occur due to storage at extreme temperatures.

5. Conclusion

The results showed all formulations of the ointment preparations have good physical criteria at the organoleptic, homogeneity, protection power, and adhesion test. As indicated by the smallest acid

Table 5. Spreadability test results (n=3, mean ± SD)

Day	F0	F1	F2	F3
1	3.17 ± 0.09	3.79 ± 0.03	3.98 ± 0.06	3.89 ± 0.06
3	3.42 ± 0.06	4.44 ± 0.02	4.23 ± 0.20	4.43 ± 0.04
7	3.46 ± 0.26	4.52 ± 0.07	4.40 ± 0.16	4.63 ± 0.13
14	3.68 ± 0.26	4.74 ± 0.11	4.55 ± 0.12	4.86 ± 0.07
21	3.92 ± 0.17	5.24 ± 0.09	4.77 ± 0.06	5.16 ± 0.10
28	4.30 ± 0.22	5.92± 0.26	5.26 ± 0.18	5.34 ± 0.06

Table 6. Acid number test results

Formula	The volume of KOH (mL) used on Day		Acid Number
	1	28	
BHA 0 % (F0)	1.7 mL	2.6 mL	10.428
BHA 0.01 % (F2)	1.7 mL	1.9 mL	7.622
BHA 0.02 % (F3)	1.7 mL	2.0 mL	8.022

number. the best formula in preventing the oxidation process is the ointment formula containing 0.01% BHA (F2). The addition of BHA can affect the stability of the ointment and can reduce the oxidation process due to a decrease in the acid number.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Murtini G. Farmasetika Dasar. Jakarta: Kementrian Kesehatan Republik Indonesia. 2016. 76 p.
2. Chasanah E. Nurilmala M. Purnamasari AR. Fithriani D. Chemical composition. albumin content and bioactivity of natural and cultivated snakeheadprotein snakehead fish (*Channa striata*) protein extract. J Pascapanen dan Bioteknol Kelaut dan Perikan. 2015;10(2):123.
3. Fatma II. Haryanti S. Widodo S. Suedy A. Uji Kualitas Madu Pada Beberapa Wilayah Budidaya Lebah Madu Di Kabupaten Pati. J Biol. 2017;6(2):58–65. Available from: https://ejournal3.undip.ac.id/index.php/biologi/article/view/19538
4. Departemen Kesehatan Republik Indonesia. Farmakope Indonesia Edisi Ketiga. Jakarta: Departemen Kesehatan Republik Indonesia; 1979.
5. Arpi N. Kombinasi Antioksidan Alami α-tokoferol dengan Asam Askorbat dan Antioksidan Sintetis BHA dengan BHT dalam Menghambat Ketengikan Kelapa Gongseng Giling (*U Neulheu*) selama Penyimpanan. J Teknol dan Ind Pertan Indones. 2014;6(2).
6. Fitri N. Butylated hydroxyanisole sebagai Bahan Aditif Antioksidan pada Makanan dilihat dari Perspektif Kesehatan. J Kefarmasian Indones. 2014;4(1):41–50.
7. Rowe RC. Shesekey PJ. Quinn M. Handbook of Pharmaceutical Excipients. 6th edition. London: Pharmaceutical Press. 2009.
8. Waney R. Gayatricitraningtyas. Abidjulu J. Pengaruh Suhu Terhadap Stabilitas Serta Penetapan Kadar Tablet Furosemida menggunakan Spektrofotometer Uv-Vis. Pharmacon. 2012;1(2):93–7.
9. Andrie M. Sihombing D. Efektivitas Sediaan Salep yang Mengandung Ekstrak Ikan Gabus (*Channa striata*) pada Proses Penyembuhan Luka Akut Stadium II Terbuka pada Tikus Jantan Galur Wistar The Effectiveness of Snakehead (*Channa striata*) Extract- Containing Ointment on Healing Proce. Pharm Sci Res ISSN Pharm Sci Res. 2017;4(2):88–101.
10. Ali F. Stevani H. Rachmawaty D. Formulasi Dan Stabilitas Sediaan Body Scrub Bedda Lotong Dengan Variasi Konsentrasi Trietanolamin. Media Farm. 2019;15(1):71.
11. Susilowati EP. Wahyuningsih SS. Optimasi Sediaan Salep yang Mengandung Eugenol dari Isolasi Minyak Cengkeh (*Eugenia caryophylatta* Thunb.). Indones J Med Sci. 2014;1(2):29–34.
12. Pratimasari D. Sugihartini N. Yuwono T. Evaluasi Sifat Fisik Dan Uji Iritasi Sediaan Salep Minyak Atsiri Bunga Cengkeh Dalam Basis Larut Air. J Ilm Farm. 2015;11(1):9–15.
13. Sandi DAD. Musfirah Y. Pengaruh Basis Salep Hidrokarbon dan Basis Salep Serap terhadap Formulasi Salep Sarang Burung Walet Putih. J Ilm Manuntung. 2018;2(2):149–55.
14. Azizah Z. Rasyid R. Kartina D. Pengaruh pengulangan dan lama penyimpanan terhadap ketengikan minyak kelapa dengan metode asam thiobarbiturat (tba). J Higea Farm. 2016;8(2):189–200.
15. Taurina W. Andrie M. Anjeli L. The gel formulation of the aqueous phase of snakehead fish (Channa striata) extract with various combinations of HPMC K4M and Carbopol 934. Pharmaciana. 2018;8(1):97.
16. Ika P DA. Sibarani J. Suaniti N. Sifat Fisikokimia Hand and Body Cream Dengan Pemanfaatan Ekstrak Etanol Bunga Gemitir (*Tagetes erecta* L.) Dan Bunga Pacar Air Merah (*Impatiens balsamina* L.) Dari Limbah Canang. Cakra Kim (Indonesian E-Journal Appl Chem. 2017;5(1):1.
17. Sandi, D. A. D., & Musfirah, Y. Pengaruh Basis Salep Hidrokarbon dan Basis Salep Serap terhadap Formulasi Salep Sarang Burung Walet Putih (*Aerodramus fuciphagus*). Jurnal Ilmiah Manuntun. 2018;4(2): 149-155
18. Khoirunnisa Z. Wardana AS. Rauf R. Angka Asam dan Peroksida Minyak Jelantah Dari Penggorengan Lele Secara Berulang. J Kesehat. 2020;12(2):81–90.
19. Andrie M, Taurina W, Ayuningtyas D. Accelerated Stability Test of Snakehead Fish Extract (*Channa striata*) and Kelulut Honey (*Heterotrigona itama*) Ointment Combination with BHT as an Antioxidant. Traditional Medicine Journal. 2022; 27(1): 12.
20. Sopianti, Densi Selpia, Herlina Herlina, and Handi Tri Saputra. Penetapan kadar asam lemak bebas pada minyak goreng. Jurnal katalisator 2.2 (2017): 100-105
21. Djuma AW. Effect Frequency Fryingonperoxide Number to Cooking Oil in Packaging. J Info Kesehat. 2014;13(2):796–803.