Borneo Journal of Pharmacy
Vol 5 Issue 2 May 2022
Page 126 – 135

http://journal.umpalangkaraya.ac.id/index.php/bjop/article/view/3321
DOI: https://doi.org/10.33084/bjop.v5i2.3321
e-ISSN: 2621-4814

Research Article

Optimization of Quercetin Gel Formulation using Factorial Design Method and Antibacterial Test against *Propionibacterium acnes*

M. Andi Chandra*

Ilham Kuncahyo

Ana Indrayati

Department of Pharmacy, Universitas Setia Budi, Surakarta, Central Java, Indonesia

*email: andychandraa1@gmail.com

Keywords:
Factorial design
Optimum formula
Propionibacterium acnes
Quercetin gel

Abstract

Quercetin is a flavonoid from a group of polyphenolic flavonoid compounds. Quercetin can be used as an alternative to acne treatment, predominantly triggered by *Propionibacterium acnes*. This study aimed to determine the effect and proportion of carbopol 940, propylene glycol, and glycerin on the physical quality of quercetin gel, the ability of the optimum formula in an antibacterial test, and its diffusion using Franz diffusion. This study uses the factorial design method for formula optimization. Optimization was carried out with the parameters of the physical quality of the gel tested, including viscosity, dispersibility, antibacterial, and Franz diffusion. The combination of carbopol 940, glycerin, and propylene glycol affected the physical quality test of quercetin gel, carbopol and glycerin significantly affected viscosity. In contrast, glycerin and propylene glycol significantly affected Franz's dispersion, antibacterial, and diffusion properties. The optimum proportion of the combination of carbopol 940, glycerin, and propylene glycol in the manufacture of quercetin gel using the factorial design method obtained a concentration of carbopol 940 of 0.5%, glycerin of 15%, and propylene glycol of 10%. The optimum formula ability in the antibacterial test was 22.20 mm, and the cumulative percent of quercetin penetrated was 97.91%.

Received: March 10th, 2022
Revised: April 11th, 2022
Accepted: May 10th, 2022
Published: May 31th, 2022

INTRODUCTION

Acne or acne vulgaris is an infection or inflammation that occurs in areas of the body that produce much oil, such as facial skin. The activation of excess oil causes acne, causing clogged pores or oil gland ducts on the facial skin and hair (pilosebaceous tract)[1]. A bacterial infection can also cause acne. The most dominant bacteria that can trigger the formation of acne is *Propionibacterium acnes*[2]. Acne treatment requires another alternative: natural compounds, such as quercetin. Quercetin is one of the flavonoids from a group of polyphenolic flavonoid compounds; quercetin is generally obtained as aglycones from several flavonoid glycosides[3]. Quercetin has a mechanism of action as an antibacterial that causes acne by inhibiting protein synthesis in bacteria and inhibiting the production of toxin metabolites in bacteria[4]. Based on the mechanism of action of quercetin, it can be used as an antibacterial treatment to facilitate its use and other considerations so that quercetin is prepared in advance.

Quercetin is a hydrophobic compound classified in the Biopharmaceutical Classification System (BCS) II, which means that quercetin has high permeability but low solubility[5]. The problem with quercetin in oral administration is that it has low bioavailability in the body, where its absorption is limited, its elimination is rapid, and it is extensively metabolized[6]. Problems that occur in quercetin gel preparations, the study's results[7] found that the largest inhibition zone was found in formula 1 with a concentration value of 0.05% w/w. In comparison, the minor inhibition zone was found in formula 3 with the highest concentration of 0.50% w/w; the higher the concentration, the greater the minimum inhibition zone obtained.

However, the results obtained in this study were contrary; the greater the concentration used, the smaller the minimum inhibition zone. This is because the phenol (-OH) group of quercetin will bind to HPMC to replace the –OH group of distilled water, resulting in decreased antibacterial inhibition[8]. So this study was further developed by using different gelling agents and different bacteria and optimizing the quercetin gel formula to obtain the optimum formula, which is expected to give better results. This study makes quercetin in gel preparations because it is to help treat acne by determining the optimum formula so that the quercetin gel preparation will be even more optimal in the composition of the formula concentration and antibacterial activity.

Transdermal dosage forms, one of which is a gel preparation. The gel is one of the dominant drug preparations used by the public[9]. Good pharmaceutical preparation must meet safety parameters but also have an optimum composition. A balanced composition between gelling agent and humectant will improve gel stability so that the gel can meet the parameters of pharmaceutical preparations that are good, effective, acceptable, and safe to use[10]. This study used carbopol as a gelling agent, propylene glycol, and glycerin as a humectant. The combination of humectants between propylene glycol and glycerin will maintain the stability of the preparation[11].

Testing the antibacterial activity of quercetin gel preparations uses the well diffusion method. The advantage of the well diffusion method is that it is easier to measure the area of the inhibition zone formed because the isolates are active not only on the top surface of the nutrient agar but also down to the bottom[12]. The well diffusion method is selected because it can accommodate a higher sample concentration than the disc method so that it diffuses faster in the media, which causes the inhibitory effect of bacteria to become stronger[13]. Based on this, it is known that quercetin compounds have problems in their bioavailability when made in oral preparations. Therefore, researchers are interested in making quercetin compounds in gel preparations, optimizing the quercetin gel formula using factorial design methods, and testing antibacterial activity against *P. acnes* bacteria. To obtain the optimum formula from the results of the physical quality test of the quercetin gel preparation and the antibacterial activity test using the well diffusion method.

MATERIALS AND METHODS

Tools and materials

The tools used in this study were analytical balance (AD-600i), micropipette, slide, microscope, hot plate (THERMO Scientific), centrifuge (HC6 Centrifuge), UV-Vis Spectrophotometry (T60), pH meter Atc, 30 viscometer NDJ-5S, incubator (ESCO Isotherm), autoclave, Laminar Air Flow (LAF), weights, and Franz cell diffusion (FDC-2C). The materials used in this study were quercetin, Carbopol 940, glycerin, propylene glycol, nipagin, nipasol, TEA, aqua PA, doxycycline antibiotics 30 m/disc, Nutrient Agar media, *P. acnes* ATCC 11827 bacteria, Mueller-Hinton Agar media, hydrogen peroxide, rabbit plasma, crystal violet, Lugol's, alcohol acetone, ethanol 96%, safranin, and phosphate buffer pH 7.4.

Sterilization of tools and materials

Equipment and materials used in the test were sterilized first. Tools such as test tubes, stirring rods, Erlenmeyer, Petri dishes, or test materials such as NA and MHA media aquadest were sterilized using an autoclave at 121°C for 15 minutes. Tools such as wire loops were sterilized by heating over a flame[14].

Media preparation

The manufacture of MHA media started by weighing 2.66 g of MHA and dissolved into an Erlenmeyer flask with distilled water until it reached a volume of 70 mL, then heated until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes. The media was poured into a petri dish of 35 mL and allowed to solidify[15].

Bacterial suspension preparation

The suspension of the *P. acnes* ATCC 11827 test colonies obtained from the hospital and laboratory equipment chemical scientific supply-General Trade, North Jakarta, was rejuvenated by taking one dose of the colony from solid NA media into

a test tube containing 5 mL of physiological NaCl. The turbidity of the test colony suspension was standardized to the 0.5 McFarland standard (approximately 1.5 x 10^8 CFU/mL). The suspension should be used as the inoculum within 15 minutes[15].

Preparation of quercetin gel

The composition of the quercetin gel is presented in **Table I**. First, the weighing was carried out according to the formula, then grinding was carried out on the powder material taken. Carbopol swelling was carried out using distilled water at 37±2°C with constant grinding (mixture 1). Quercetin was dispersed into propylene glycol and then mixed until homogeneous (mixture 2). Nipagin and nipasol were dispersed into glycerin and mixed until homogeneous (mixture 3). Mixtures 2 and 3 were mixed and stirred using a stirrer (mixture 4). mixture 4 was added little by little into Mixture 1 until homogeneous by grinding. Distilled water was added until 100 mL. TEA was added drop by drop while grinding slowly until a homogeneous gel with good consistency was formed. The quercetin gel was evaluated, including organoleptic examination, homogeneity, viscosity, spreadability, pH, antibacterial, and Franz diffusion test[7].

Table I. Composition of Carbopol 940, glycerin and propylene glycol

Formula	Composition (%)		
	Carbopol 940	Glycerin	Propylene glycol
1	0,5	15	5
2	2	5	5
3	2	5	10
4	0,5	15	10
5	0,5	5	5
6	2	15	10
7	0,5	5	10
8	2	15	5

Physical quality check of quercetin gel

Organoleptic and homogeneity test

An organoleptic test was carried out by observing the quercetin gel preparation, including color, odor, and shape. The homogeneity test was carried out using a certain amount of preparations smeared on glass pieces. Observations were made on the preparations: the preparations had to show a homogeneous arrangement, and no coarse grains were seen; the homogeneity test was repeated three times[16].

Viscosity test

A viscosity test was carried out by adding 15 g of gel to the container. The test was carried out using a Viscometer NDJ-5S with rpm 30 and spindle No 4. The viscosity results were observed. The procedure was repeated three times. Good viscosity ranged between 3,000-50,000 cPs[17].

Spreadability test

The spreadability/dispersion test was carried out after 24 hours of manufacture. This test is carried out by taking 0.5 g of the gel preparation and placing it in the middle of a round glass that has been given a scale. Then covered with glass as the initial load and allowed to stand for a minute. After a minute of adding a load of 50 g, the diameter of the dispersion was measured. The load was then added 50 g and allowed to stand again for a minute, and the exact measurements were carried out as before for each addition of 50 g of load to 250 g, repeated three times[18].

pH test

The pH test was carried out using a pH meter by dissolving 1 g of the gel preparation in 10 mL of aquadest. The pH meter before use was calibrated using acetate buffer pH 4.0 and phosphate buffer pH 7.0. The calibrated pH meter was ready for use by dipping it into the gel. The pH value on the device was recorded, and repetitions were performed three times. The pH value of the gel preparation must be in the range of a neutral pH or suitable for the skin, between 4.5-6.5[19].

Antibacterial test

A suspension of 100 L of *P. acnes* bacteria was inoculated into six Petri dishes containing MHA media, then leveled on the media using L rods. Wells were made using a cork borer. 70 mg of gel was inserted into the wells that had been made; the holes were in the media with a diameter of 6 mm, and the size of the petri dish was 11 cm. One petri dish consisted of four wells for four formulas of the quercetin gel preparation; a total of six Petri dishes and two Petri dishes for positive control and negative control were then incubated for 24 hours at 37°C. The clear inhibition zone around the well was observed and measured using a caliper. The procedure was repeated three times[20].

Determination of the optimum formula for quercetin gel

The optimum formula was determined after testing the gel preparation's physical quality: viscosity, dispersibility, antibacterial, and *in vitro* penetration tests using Franz diffusion cells. The results of the physical quality of the gel preparation were then processed using expert design software using the factorial design method. The optimum formula selected was expected to have a desirability value close to 1. The optimum formula for the resulting quercetin gel was expected to have a suitable viscosity value of 3,000-50,000 cPs. The spreadability of a good gel preparation was 5-7 cm[11]. The antibacterial value in the strong category was in the range of values of 11-20 mm. *In vitro* penetration of Franz diffusion results reaches 40-90%[21].

RESULTS AND DISCUSSION

Organoleptic test

Organoleptic observations were seen from the gel preparation's shape, color, and odor, and the results obtained were recorded. The organoleptic results of eight quercetin gel formulas can be seen in **Figure 1** and **Table II**. Purpose organoleptic examination was carried out to determine the physical properties of the gel-based on the results of direct visual observation. Research conducted by Irianto *et al*.[22] related to organoleptic tests also observed the gel preparations made, including the gel preparation's shape, color, and odor. This test needs to be done because it relates to the convenience of use as a topical preparation. Observation of the homogeneity of the eight quercetin gel formulations gave good results, which looked homogeneous. Purgiyanti and Pratiwi[23] also reported that the gel homogeneity test was carried out to determine the mixing of each component in the gel manufacture.

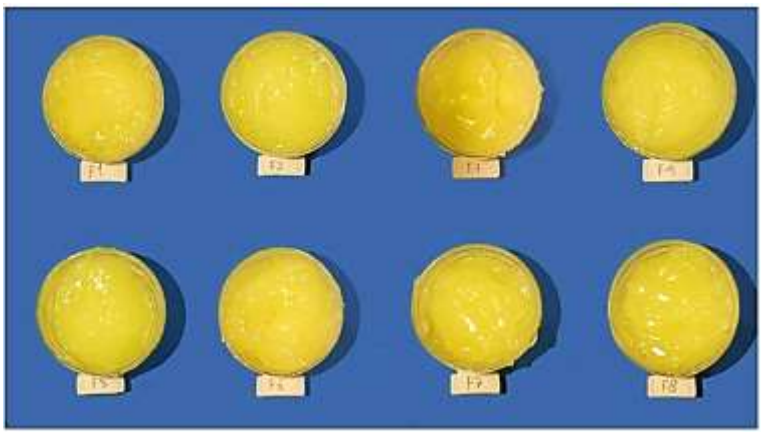

Figure 1. Quercetin gel preparation

pH test

The pH test in this study aims to determine whether the gel produced is acceptable for skin pH because it can cause skin irritation if not in accordance with skin pH. The results of the pH test for quercetin gel preparations can be seen in **Table III**. According to Gozali *et al*.[24], the skin's pH balance ranges from 4.5 to 6.5 because if the pH value is less than 4 and more than 7, it is feared that it can irritate the skin. In normal skin with balanced sebum secretion, the pH of facial skin is at 5.5. Meanwhile, oily skin tends to be more acidic, resulting in a lower pH, often owned by people with acne-prone and easily

irritated skin. The results of the pH test in this study were by the requirements if used on normal skin or acne-prone skin because the pH range obtained was still in the pH range of 4.5-6.5[11].

Table II. Organoleptic examination of quercetin gel

Formula	Shape	Color	Odor
1	Soft thick	Yellow	Typical
2	Soft thick	Yellow	Typical
3	Soft thick	Yellow	Typical
4	Soft thick	Yellow	Typical
5	Soft thick	Yellow	Typical
6	Soft thick	Yellow	Typical
7	Soft thick	Yellow	Typical
8	Soft thick	Yellow	Typical

Table III. pH examination of quercetin gel

Formula	pH±SD
1	5.6±0.05
2	5.5±0.20
3	5.8±0.05
4	5.6±0.10
5	5.6±0.15
6	5.7±0.15
7	5.6±0.15
8	5.6±0.10

Antibacterial test

The quercetin gel antibacterial activity test was carried out using the well diffusion method. The pitting method was chosen because this method can accommodate a higher gel concentration than the disc method. So that it diffuses more quickly in the media, which causes the inhibitory effect of bacteria to be stronger. The antibacterial activity test of quercetin gel can be seen in **Table IV**. The components that influence the antibacterial activity in this study are glycerin and propylene glycol. Glycerin has a preservative for antimicrobials if used in concentrations <20%; therefore, glycerin influences the results of antibacterial tests. Glycerin can also increase the active substance's permeability to increase the gel preparation's antibacterial inhibition[25]. According to Anastasia *et al*.[26], the addition of glycerin in the formula affects the activity of the inhibitory zone of the active substance, which can be seen from the comparison of the diameter of the inhibition zone in each formula. Zhang *et al*.[27] also stated that propylene glycol facilitates mixing between quercetin and gel base, so the higher the propylene glycol concentration is used, the antibacterial activity will increase. Propylene glycol has lipophilic properties, so it will help quercetin to penetrate the cell wall of bacteria. Propylene glycol has a synergistic effect helping the penetration of the active substance and works as an antimicrobial component.

Table IV. Antibacterial activity of quercetin gel

Formula	Inhibition zone±SD (mm)	Antibacterial activity category
1	18.88±0.06	Strong
2	15.47±0.44	Strong
3	15.79±0.72	Strong
4	18.68±0.05	Strong
5	17.39±0.02	Strong
6	16.62±0.02	Strong
7	17.66±0.02	Strong
8	16.42±0.27	Strong

Determination of the optimum formula for quercetin gel

Propionibacterium acnes grow optimally at a pH of 6-7[28]; the preparations obtained a pH of around 5. This causes the bacteria not to grow optimally because the pH is too acidic. This condition can increase the inhibitory power of the gel preparation, which is made more significant. In general, gel viscosity influences the inhibition of bacteria. This is because the high viscosity will inhibit the release of the active substance quercetin[29]. However, in this study, the large viscosity did not affect the release of quercetin because it was assisted by propylene glycol and glycerin. On the contrary, it would increase the

contact time of the gel with the skin, thereby increasing the antibacterial inhibition. The calculation results of the factorial design response to the value of viscosity, dispersion, antibacterial, and diffusion Franz can be seen in **Table V**. The equations presented arranged based on the factorial design chart for the response values of viscosity, spreadability, antibacterial, and Franz diffusion. Based on these data, it can be observed that the value of each parameter obtained varies with each formula. There are differences in these values. It was caused by Carbopol 940, glycerin, and propylene glycol levels.

Table V. Calculation of the factorial design response

Response	Factorial design equation
Viscosity	Y= +2191.50 (A) +0.6100 (B)- 66.480 (C)
Spreadability	Y= -0.3500 (A) +0.0175 (B)+0.04500 (C)
Antibacterial	Y= -1.385 (A) +0.107 (B) +0,029 (C)
Franz diffusion	Y= -15.17 (A) +0.880 (B)+0.549 (C)

In the equation generated from the viscosity response, it is known that the most influential factors in changing the viscosity value are Carbopol 940 and glycerin. The interaction between Carbopol 940 and glycerin components in each formula has a significant effect. The mechanism of gel formation by Carbopol 940 is by binding the solvent to the structure of the carbopol polymer so that crosslinks occur in the polymer, causing water to be trapped in it. When carbopol comes into contact with the water environment, anionic polyelectrolytes are formed, which then form a hydrogel structure[30]. When TEA is added, the negative charge repulsion along the polymer chain will increase, and the osmotic pressure inside the expanding polymer causes the formation of a three-dimensional structure. In other words, carbopol macromolecules combine to form flocks in solution. Increasing the polymer concentration in the aqueous environment will increase crosslinked polymer chains after adding the base agent. The higher the polymer concentration in the aqueous environment, the resulting gel network is denser and more compact so that the viscosity will increase[31]. According to the results obtained, glycerin affects viscosity. The mechanism of glycerin in increasing viscosity is by increasing the surface tension of the gel[32].

In the equation generated from the spreadability response, it is known that glycerin and propylene glycol are the most influential factors in changing the dispersion value. The interaction between the two components of glycerin and propylene glycol in each formula has a significant effect. The positive value of the equation is found in the components of glycerin and propylene glycol, which indicates that these two components affect the spreadability of the quercetin gel preparation. Semi-solid preparations will experience an increase in the number of dissolved particles caused by glycerin and propylene glycol. This will increase the interaction of the particles so that the movement of molecules in the preparation increases the spread of the gel[33]. If preparation has a high spreadability, the active substance will be evenly distributed and more effective in producing a therapeutic effect[34].

In the equation generated from the Franz diffusion response, it is known that glycerin and propylene glycol are the most influential factors in changing the dispersion value. Based on this equation, the value of the glycerin and propylene glycol components showed positive results, which indicated that the two components affected the percent penetration value of quercetin. Glycerin and propylene glycol cause dehydration of the skin membrane. This will increase the active substance's permeability from formulations with a water base[35].

The Design-Expert version 13 program will select a formula with the highest desirability so that the selected optimum formula will produce the desired physical properties of the gel. A good desirability value is close to 1. The Superimposed quercetin gel contour plot preparation is shown in **Figure 2**. Based on the contour plot in the figure, the optimum point is obtained, which is marked by a box labeled prediction accompanied by a given desirability value of 0.928.

The results of the physical properties test of the optimum quercetin gel formula were compared with the predicted values obtained from the Design-Expert program by statistical analysis using a one-sample t-test. The calculation is intended to determine the difference between the experimental value of the optimum gel formula and the predicted results from the program. These results indicate that the resulting formula has physical properties that match the predictions. All parameters of physical properties have a significance value greater than 0.05, so it can be concluded that the comparison of program prediction data with experimental values of the optimum quercetin gel formula is not significantly different. The optimum

formula composition obtained from the factorial design software shows that the proportion of the optimum formula concentration is carbopol 940 0.5%, glycerin 15%, and propylene glycol 10%. A comparison of the predicted value with the experimental value of the optimum formula can be seen in **Table VI**.

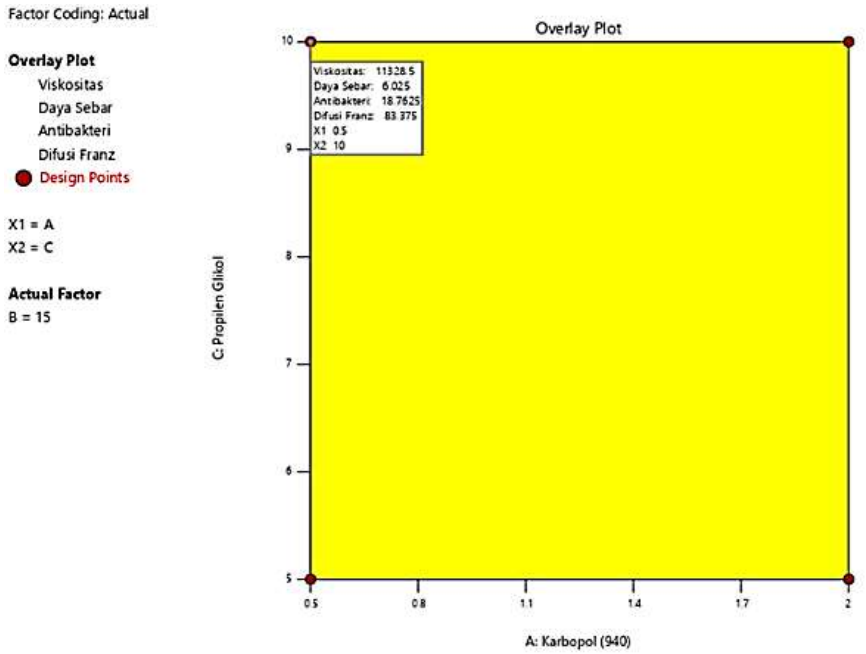

Figure 2. Superimposed quercetin gel contour plot. (Translation notes: *Viskositas*: viscosity; *Daya sebar*: spreadability; *Antibakteri*: antibacterial; *Difusi Franz*: Franz diffusion)

Table VI. Comparison of the predicted value with the experimental value of the optimum formula

Physical quality	Predictive value	Trial value	Sig.
Viscosity (cPs)	11,328	14,980	0.088*
Dispersibility (cm)	6.02	7.20	0.057*
Antibacterial/inhibition zone (mm)	18.76	22.20	0.053*
Franz diffusion (%)	83.37	97.91	0.051*

* Not significantly different

A one-sample t-test with a 95% confidence level was used to compare the predicted and experimental values. In the viscosity response, a value of >0.05 was obtained, so it was concluded that there was no significant difference between the predicted and experimental values. In the dispersion response, a value of >0.05 was obtained, concluding that there was no significant difference between the predicted and experimental values. In the antibacterial response, a value of >0.05 was obtained, concluding that there was no significant difference between the predicted and experimental values. In the Franz diffusion response, a value of >0.05 was obtained, concluding that there was no significant difference between the predicted and experimental values. It can be concluded that the results of the overall one sample t-test between the Franz diffusion predictions and the experimental values are not significantly different, indicating that the experimental values are close to the software predictions because the sign values of all tests are >0.05.

CONCLUSION

The factorial design equations obtained from the response to the viscosity, dispersibility, antibacterial, and Franz diffusion tests can be concluded that the combination of carbopol 940, glycerin, and propylene glycol has a significant effect. The optimum formula composition from the predicted factorial design is carbopol 940 of 0.5%, glycerin 15%, and propylene glycol 10%. Moreover, the optimum formula ability of quercetin gel against antibacterial test has an inhibition zone value of 22.20 mm and penetration of the active substance quercetin with a cumulative percentage of penetrated quercetin in the gel

preparation of 97.91%. Comparison of the results of the predicted value and the experimental value of the physical quality test of the quercetin gel, it can be concluded that the overall results of the test using the one-sample t-test between the predicted value and the experimental value were not significantly different, because the sign values of all tests were >0.05.

ACKNOWLEDGMENT

The author would like to thank and support the Master of Pharmacy study program at Setia Budi University, and thanks also to the Laboratory of Pharmacy-Microbiology Technology, University of Sari Mulia Banjarmasin where the research was conducted. This research did not obtain external funding.

AUTHORS' CONTRIBUTION

M. Andi Chandra: research team leader, validation, and article writing. **Ilham Kuncahyo**: supervision, validation, methodology. **Ana Indrayati**: supervision, methodology.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The author declares there is no conflict of interest and equivalent.

REFERENCES

1. McLaughlin J, Watterson S, Layton AM, Bjourson AJ, Barnard E, McDowell A. Propionibacterium acnes and Acne Vulgaris: New Insights from the Integration of Population Genetic, Multi-Omic, Biochemical and Host-Microbe Studies. Microorganisms. 2019;7(5):128. doi:10.3390/microorganisms7050128

2. Platsidaki E, Dessinioti C. Recent advances in understanding Propionibacterium acnes (Cutibacterium acnes) in acne. F1000Res. 2018;7:F1000 Faculty Rev-1953. doi:10.12688/f1000research.15659.1

3. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Medicines. 2018;5(3):93. doi:10.3390/medicines5030093

4. Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A. Review on the Antibacterial Mechanism of Plant-Derived Compounds against Multidrug-Resistant Bacteria (MDR). Evid Based Complement Alternat Med. 2021;2021:3663315. doi:10.1155/2021/3663315

5. Truzzi F, Tibaldi C, Zhang Y, Dinelli G, Amen ED. An Overview on Dietary Polyphenols and Their Biopharmaceutical Classification System (BCS). Int J Mol Sci. 2021;22(11):5514. doi:10.3390/ijms22115514

6. Riva A, Ronchi M, Petrangolini G, Basisio S, Allegrini P. Improved Oral Absorption of Quercetin from Quercetin Phytosome®, a New Delivery System Based on Food Grade Lecithin. Eur J Drug Metab Pharmacokinet. 2019;44:169-77. doi:10.1007/s13318-018-0517-3

7. Herslambang RA, Rahmawanty D, Fitriana M. Herslambang aktivitas sediaan gel kuersetin terhadap staphylococcus epidermidis the activity of quersetin gel againts Staphylococcus Epidermidis. J Farmasi Galenika Galenika J Pharm. 2015;1(1):59–64. doi: https://doi.org/10.22487/j24428744.2015.v1.i1.7901

8. Shamsudin NF, Ahmed QU, Mahmood S, Shah SAA, Khatib A, Mukhtar S, et al. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. Molecules. 2022;27(4):1149. doi:10.3390/molecules27041149

9. Cheng YC, Li TS, Su HL, Lee PC, Wang HMD. Transdermal Delivery Systems of Natural Products Applied to Skin Therapy and Care. Molecules. 2020;25(21):5051. doi:10.3390/molecules25215051

10. Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The Optimization of Gel Preparations Using the Active Compounds of Arabica Coffee Ground Nanoparticles. Sci Pharm. 2019;87(4):32. doi:10.3390/scipharm87040032

11. Sayuti NA. Formulasi dan Uji Stabilitas Fisik Sediaan Gel Ekstrak Daun Ketepeng Cina (Cassia alata L.). J Kefarmasian Indones. 5(2):74–82. doi:10.22435/jki.v5i2.4401.74-82

12. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56. doi:10.1016/j.ijantimicag.2005.09.002

13. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-9. doi:10.1016/j.jpha.2015.11.005

14. Yunus R, Mongan R, Rosnani. Cemaran Bakteri Gram Negatif pada Jajanan Siomay di Kota Kendari. Med Lab Technol J. 2017;3(1):87-92.

15. Nurhayati LS, Yahdiyani N, Hidayatulloh A. Perbandingan Pengujian Aktivitas Antibakteri Starter Yogurt dengan Metode Difusi Sumuran dan Metode Difusi Cakram. J Teknologi Hasil Peternakan. 2020;1(2):41-6. doi:10.24198/jthp.v1i2.27537

16. Panjaitan EN, Saragih A. Formulasi Gel Dari Ekstrak Rimpang Jahe Merah (Zingiber officinale Roscoe). J Pharm Pharmacol. 2012;1(1):9–20.

17. Pertiwi RD, Kristanto J, Praptiwi GA. Uji Aktivitas Antibakteri Formulasi Gel Untuk Sariawan Dari Ekstrak Daun Saga (Abrus precatorius Linn.) terhadap Bakteri Staphylococcus aureus. J Ilmiah Manuntung. 2016;2(2):239-47. doi:10.51352/jim.v2i2.72

18. Emelda E. Formulasi dan uji sifat fisik sediaan gel tunggal dan kombinasi ekstrak etanolik daun sirih merah (Pipper crocatum) minyak kayu manis (Cinnamon oil). Inpharnmed J Indones Pharm Nat Med J. 4(2):43-53. doi:10.21927/inpharnmed.v4i2.1405.

19. Zulkarnain AK., Marchaban, Wahyuono S, Susidarti RA. Pengaruh konsentrasi mahkota dewa terhadap stabilitas lotion – krim serta uji tabir surya secara effect lotion – cream phaleria macrocarpa. Majalah Farmaseutik. 11(3):328–35. doi:10.22146/farmaseutik.v11i3.24124

20. Julianti E, Rajah KK, Fidrianny I. Antibacterial Activity of Ethanolic Extract of Cinnamon Bark, Honey, and Their Combination Effects against Acne-Causing Bacteria. Sci Pharm. 2017;85(2):19. doi:10.3390/scipharm85020019

21. Winastri NLAP, Muliasari H, Hidayati E. Aktivitas Antibakteri Air Perasan Dan Rebusan Daun Calincing (Oxalis corniculata L.) terhadap Streptococcus mutans. Berita Biol. 2020;19(2):223-30. doi:10.14203/beritabiologi.v19i2.3786

22. Irianto IDK, Purwanto, Mardan MT. Aktivitas Antibakteri dan Uji Sifat Fisik Sediaan Gel Dekokta Sirih Hijau (Piper betle L.) Sebagai Alternatif Pengobatan Mastitis Sapi. Majalah Farmaseutik. 2020;16(2):202-10. doi:10.22146/farmaseutik.v16i2.53793

23. Purgiyanti, Pratiwi RI. Pembuatan gel antinyeri dari minyak atsiri bunga cengkeh. Parapemikir J Ilmiah Farmasi. 2019;8(1):72–5. doi:10.30591/pjif.v8i1.1305

24. Gozali D, Abdassah M, Subghan A, Al Lathiefah S. Formulasi krim pelembab wajah yang mengandung tabir surya nanopartikel zink oksida salut silikon. Farmaka. 2009;7(1):37–47.

25. Stout EI, McKessor A. Glycerin-Based Hydrogel for Infection Control. Adv Wound Care. 2012;1(1):48-51. doi:10.1089/wound.2011.0288

26. Anastasia A, Yuliet, Tandah MR. Formulasi Sediaan Mouthwash Pencegah Plak Gigi Ekstrak Biji Kakao (Theobroma cacao L) Dan Uji Efektivitas Pada Bakteri Streptococcus mutans. J Farmasi Galenika Galenika J Pharm. 2017;3(1):84-92. doi:10.22487/j24428744.2017.v3.i1.8144

27. Zhang Z, Li J, Ma L, Yang X, Fei B, Leung PHM, et al. Mechanistic Study of Synergistic Antimicrobial Effects between Poly (3-hydroxybutyrate) Oligomer and Polyethylene Glycol. Polymers. 2020;12(11):2735. doi:10.3390/polym12112735

28. Bossard DA, Ledergerber B, Zingg PO, Gerber C, Zinkernagel AS, Zbinden R, Achermann Y. Optimal Length of Cultivation Time for Isolation of Propionibacterium acnes in Suspected Bone and Joint Infections Is More than 7 Days. J Clin Microbiol. 2016;54(12):3043-9. doi:10.1128/jcm.01435-16

29. An SH, Ban E, Chung IY, Cho YH, Kim A. Antimicrobial Activities of Propolis in Poloxamer Based Topical Gels. Pharmaceutics. 2021;13(12):2021. doi:10.3390/pharmaceutics13122021

30. Safitri FI, Nawangsari D, Febrina D. Overview: Application of Carbopol 940 in Gel. In: Davis D, Atf G, McGowan L, bin Said I, Rahman T, Wantonoro, et al., editors. Proceedings of the International Conference on Health and Medical Sciences (AHMS 2020); 2020 Jul 18; Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia. Paris: Atlantis Press; 2020. pp.80-4. doi:10.2991/ahsr.k.210127.018

31. Ghebremedhin M, Seiffert S, Vilgis TA. Physics of agarose fluid gels: Rheological properties and microstructure. Curr Res Food Sci. 2021;4:436-48. doi:10.1016/j.crfs.2021.06.003

32. Zhang H, Grinstaff MW. Recent advances in glycerol polymers: chemistry and biomedical applications. Macromol Rapid Commun. 2014;35(22):1906-24. doi:10.1002/marc.201400389

33. Fauzee AFB, Walker RB. The impact of formulation variables on the optimization of pilot scale clobetasol 17-propionate creams. Cogent Eng, 2020;7(1):1804713. doi:10.1080/23311916.2020.1804713

34. Khan AW, Kotta S, Ansari SH, Sharma RK, Kumar A, Ali J. Formulation development, optimization and evaluation of aloe vera gel for wound healing. Pharmacogn Mag. 2013;9(Suppl 1):S6-10. doi:10.4103/0973-1296.117849

35. Björklund S, Engblom J, Thuresson K, Sparr E. Glycerol and urea can be used to increase skin permeability in reduced hydration conditions. Eur J Pharm Sci. 2013;50(5):638–45. doi:10.1016/j.ejps.2013.04.022