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RESEARCH ARTICLE

Evaluation of Physical Stability of Phaleria Macrocarpa's Leaf Extract Formulated in Nanoemulgel

Rosa Pratiwi*, Anggun Feranisa, Elsa Yuliana Sukmawati*****
*Department of Periodontics, Faculty of Dentistry, Sultan Agung Islamic University
**Department of Oral Biology, Faculty of Dentistry, Sultan Agung Islamic University
***Undergraduate Student, Faculty of Dentistry, Sultan Agung Islamic University

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ABSTRACT

Background: *Phaleria macrocarpa's leaf contains alkaloids, saponins, phenols, tannins, and flavonoids which have antioxidant, anti-inflammatory, and antibacterial activities. The nanoemulgel preparation was chosen because it can penetrate well and quickly into the mucosa thereby increasing absorption. Physical stability tests need to be carried out to ensure the quality, safety and benefits of the gel meet the expected specifications and stable during storage.* ***Objective:*** *This study aims to determine which concentration has the most stable physical stability of pH and viscosity which were observed for 28 days.* ***Materials and Methods:*** *This was an experimental laboratory design, with 30 preparation divided into 3 groups, group of 30%, 40%, and 50% concentration preparation of nanoemulgel leaves of Phaleria macrocarpa. which were tested for pH tests and viscosity tests for 28 days which was observed every 7 days in climatic chamber. The pH and viscosity tests among groups were compared and analyzed by the Repeated Anova and Pos Hoc test.* ***Results:*** *Average pH test and viscosity test 30% concentration with the average baseline and h+28 pH values were 5,02 and 4,97 and the baseline and h+28 viscosity values were 1610 cPas and 1520 cPas. The results of the average concentration of 50% pH value at baseline = 4,65 and h+28 = 4,60. The mean baseline viscosity value=1290 cPas and h+28=1170 cPas. The results of the post-Wilcoxon test showed p>0.05 in the 30% concentration group for pH and viscosity tests showed that there was no significant difference in pH and viscosity values at a concentration of 30%, It means the pH value and viscosity of the 30% concentration preparation of nanoemulgel were relatively constant from baseline to h+28.* ***Conclusion:*** *It can be concluded that the pH and viscosity test of the preparation of nanoemulgel leaves of Phaleria macrocarpa at a concentration of 30% was the most stable pH and viscosity compared to concentrations of 40% and 50%.*

Keywords: *Phaleria macrocarpa's leaf, nanoemulgel, physical stability, pH test, viscosity test.*

Correspondence: *Rosa Pratiwi, Faculty of Dentistry, Sultan Agung Islam University, Jln. Kaligawe KM 4 Semarang 50012 ph. (024) 6583584 fax. (024) 6594366 rosapratiwi@unissula.ac.id*

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INTRODUCTION

Bacterial plaque on the tooth surface is the main cause of periodontal disease. Plaque is a thin biofilm layer containing a variety of pathogenic microorganisms. The bacterium Staphylococcus aureus is one of the causes of periodontal disease. The growth of the pathogen Staphylococcus aureus, which exacerbates dental disease by forming biofilms, must be regulated.[1]

Periodontal disease treatment can be divided into surgical and nonsurgical treatment. One of nonsurgical treatment in periodontal disease is a local antibacterial agent, like metronidazole gel.[2] Metronidazole is effective for anaerobic bacteria. According to Setiawan et al., (2013) using 25% metronidazole gel is effective for reducing inflammation of periodontal disease. But, the long term use of metronidazole can cause bacterial resistance to antibiotics.[4]

Natural ingredients from Phaleria macrocarpa's leaf can be used as an antibacterial agent. Phaleria macrocarpa's leaf contains saponins, alkaloids, tannins, phenols, and flavonoids which have been shown to inhibit the growth and development of bacteria, such as Staphylococcus aureus.[4] Along with the development of science and technology, preparations of nanoemulgel widely used recently. Nanoemulgel is an emulsion preparation with a particle size of 1-100 nm suspended in a hydrogel. The smaller the particle size, the better the penetration and increase the absorption and antibacterial activity.[5,6]

Preparations made into nanoemulgel, where the gel component, type, and concentration of the added material will affect the stability of the gel. Stability is the ability of an active substance or preparation to survive within the specified specifications during the storage period[6,7]. Physical stability test aims to ensure the quality, safety, and efficacy of the gel meets the expected specifications and is stable during storage. The good physical properties of the preparation will affect the pharmacological effect.[9.10] There are several types of physical stability tests, one example of which is the pH test and the viscosity test. The physical properties of the preparation can affect the pharmacological effect. Topical preparations must have a pH that is compatible with the pH of the mucosa so as not to cause irritation and preparations with good viscosity make the preparation spread thoroughly when applied to the mucosa so that it will provide a good pharmacological effect because the active ingredients can provide an optimal effect.[11]

This study is a continuation of previous research that examined the effectiveness of nanoemulgel of extract Phaleria macrocarpa's leaves 30%, 40%, and 50% in reducing Staphylococcus aureus's biofilm thickness[17]. This study aims to determine which concentration has the most stable physical stability of pH and viscosity which were observed for 28 days.

METHODS

This is an experimental laboratory study. The number of research samples used was 30 samples, calculated using the Federer formula. The samples were divided into 3 groups, a group of Phaleria macrocarpa's leaves with concentrations of 30%, 40%, and 50%. Each group contained 10 samples. The results of this study were tested by the Shapiro Wilk test for normality. The results of the normality test were normally distributed then the data was tested by parametric statistical test analysis Repeated Anova test and Post Hoc Bonferroni. Besides, the data were not normally distributed then continued with the Friedman test and Post Hoc Wilcoxon.

The study began with the application of ethical clearance first and passed the ethics code number 337/B.1-KEPK/SA-FKG/XII/2021. Extract of Phaleria macrocarpa's leaves began with a drying and pulverizing process to become a powder, then continued with the maceration stage using 95% ethanol and converted into nanoemulgel preparations. The preparations of

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nanoemulgel of Phaleria macrocarpa's leaves were divided into 3 types of concentration, then stored in a climatic chamber at a temperature of 40ºC, then tested for physical stability in the form of a pH test and a viscosity test for the nanoemulgel preparation once a week for 28 days. Tested on day 0 before storage in the climatic chamber as a baseline.

The pH test was carried out using the Hanna HI8424 pH meter which aims to determine the degree of acidity of the preparation by inserting the electrode from the pH meter that has been rinsed with distilled water into the nanoemulgel preparation container, then observing the screen showing the most stable pH value.

The viscosity test aims to determine the value of the viscosity of a preparation using a Rion viscometer VT-06. Tests were carried out on 100 mL preparations by inserting spindle no. 2 until the entire preparation was submerged, which had previously been ensured that the rotor on the viscometer could rotate. After that, it is observed and recorded the needle of the viscometer which leads to the viscosity scale.

RESULTS

This study aims to determine the optimization of the formulation of nanoemulgel of Phaleria macrocarpa's leaves with a concentration of 30%, 40% and 50% which has good physical properties and stability.

The stability value of the preparation in this study was seen based on the pH and the viscosity test. The pH test was showed in figure 1 below:

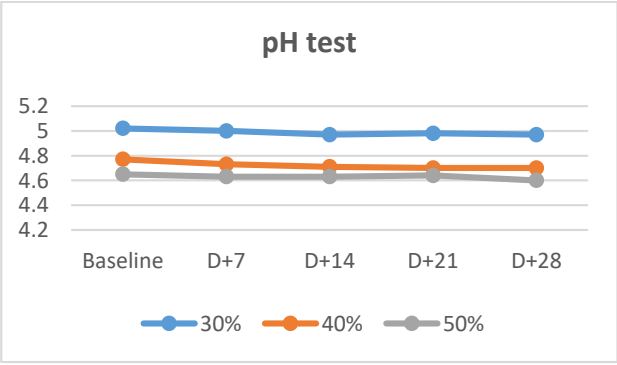

Figure 1. pH test results

Based on Figure 1 that 50% concentration group on day 28 showed the lowest average pH. which was 4.60. Meanwhile, the 30% concentration group at baseline had the highest average pH value of 5.02. From the comparison of pH values among groups. The 30% concentration group showed relatively stable pH test results from baseline to day 28.

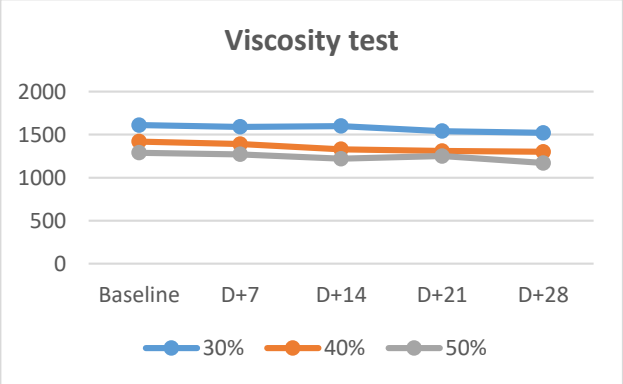

Figure 2. Viscosity test results

Based on figure 2 the viscosity value from the Rion viscometer, the lowest mean viscosity value in the 50% concentration group was on day 28, while the highest average value was in the 30% concentration group at baseline. The 30% concentration group showed relatively stable viscosity test results from baseline to day 28.

Normality test was carried out using the Saphiro-Wilk method with the results of the 30% concentration group value in the pH test and viscosity test, some data were not normally distributed ($p < 0.05$) except on day 28 ($p = 0.56$). Meanwhile, the concentration groups of 40% and 50% in the pH test and viscosity test showed that all data were normally distributed ($p > 0.05$).

The results of the Shapiro-Wilk normality test in the 30% concentration group pH test resulted in data not being normally distributed so that a non-parametric Friedman test was carried out. Friedman test obtained p value = 0.274 so that p-value> 0.05 which means there was no significant difference in pH value at a concentration group of 30% for 28 days of storage.

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In the 40% and 50% concentration groups, the results of the data were normally distributed, then the parametric test Repeated Measure Anova was carried out. The 40% concentration group obtained p value = 0.055 so that the p value> 0.05 which means that there was no significant difference in pH value in the 40% concentration group for 28 days of storage. While the 30% concentration group obtained p value = 0.030 so that the p-value <0.05 and then continued with the post hoc test.

Table 1. p-value post hoc Wilcoxon pH test concentration 30%

	baseline and D+7	D+7 and D+14	D+14 and D+21	D+21 and D+28	D+28 and baseline
p-value	0,342	0,262	0,919	0,623	0,284

Table 2. p-value post hoc test Bonferroni pH test concentration 50%*significant (p>0,05)

	Baseline	D+7	D+14	D+21	D+28
Baseline	-	0,861*	1,000*	1,000*	0,033*
D+7	-	-	1,000*	1,000*	0,475*
D+14	-	-	-	1,000*	0,018
D+21	-	-	-	-	0,082*
D+28	-	-	-	-	-

*significant (p>0,05)

Based on table 2, it was obtained that the 50% concentration pH data had a p-value <0.05, which means that there was a significant difference in pH value for 28 days of storage in the 50% concentration group of Phaleria macrocarpa leaf nanoemulgel.

Based on the results of the Shapiro-Wilk normality test, the viscosity test of the 30% concentration group had data that were not normally distributed, so a non-parametric Friedman test was carried out and the result was p value = 0.062 (p > 0.05). Meanwhile, the viscosity concentration of 40% and 50% were carried out by Repeated Measure Anova test and obtained p-value = 0.078 (p > 0.05) and p-value = 0.043 (p < 0.05). It can be interpreted that there was no significant difference in viscosity values during 28 days of storage in groups 30% and 40%. Meanwhile, the 50% concentration group continued with the Bonferroni post hoc test.

Table 3. p-value post hoc test Bonferroni viscosity test concentration 50%

Time	Baseline	D+7	D+14	D+21	D+28
Baseline	-	1,000*	0,445*	1,000*	0,030
D+7	-	-	1,000*	1,000*	0,038
D+14	-	-	-	1,000*	1,000*
D+21	-	-	-	-	0,224*
D+28	-	-	-	-	-

*significant (p>0,05)

The results of Bonferroni's post hoc p-value in the 50% concentration group viscosity test had a p-value <0.05, which means there was a significant difference in the viscosity value in the 50% concentration group for 28 days of storage.

DISCUSSION

Nanoemulgel is an emulsion preparation with a droplet size of 1-100 nm which is suspended in a hydrogel. The smaller the particle size, the easier to penetrate the skin or mucosa membrane barrier so that gives better results.[5] This study is a continuation of previous research that examined the effectiveness of nanoemulgel of extract Phaleria macrocarpa's leaves 30, 40 and 50% in reducing the thickness of Staphylococcus aureus's biofilm.[17] This study aims to determine which concentration has the most stable physical stability of pH and viscosity.

This study showed that there was no significant difference in pH tests in groups of 30% and 40% concentration during 28 days of storage. Figure 1 showed a group of 30% showed relatively stable pH test results from baseline to day 28. It means that the group of 30% had the most stable pH stability among the other group. A group of 50% showed the lowest

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pH result. In this study, the higher the extract of Phaleria macrocarpa's leaves added, the lower the pH value. According to Imanto et al (2019) showed the higher the concentration of the active ingredient added, the lower the resulting pH. The decrease in pH results can be caused by some factors such as active ingredients, the addition of preservatives, high temperature and microbial activity.

The active ingredient contained in Phaleria macrocarpa's leaves is phenol, where the decomposition of the phenol group in polyphenolic compounds can cause the amount of H+ to increase so that the pH of the preparation decreases[10]. In addition, the type of gel base used is carbopol 940 which has acidic properties and a reaction can occur between the carboxylate group on carbopol with water to form H3O+ so that it can make the gel preparation more acidic.[11]

The decrease in pH and acidic pH can also be influenced by the lack of addition of preservatives, namely methyl parabean, hydrolysis of cations from TEA as a weak base, high storage temperatures such as 40ºC, light from outside, and the formation of weak acids by microbial activity.[12,13]

Viscosity is the resistance of a liquid to flow. The higher the resistance, the higher viscosity value. The normal viscosity value range from 1000 to 2000 cPas. The average viscosity value of the 30%, 40%, and 50% concentration groups decreased after being stored at 40ºC in the climatic chamber. The decrease in viscosity value can be influenced by the concentration of the gel base used, where the higher the concentration of the gel base, the higher the viscosity of the preparation. The increase in the gelling agent can strengthen the gelling matrix so that it can increase the viscosity of nanoemulgel preparations.[14] Therefore, in this study a concentration of 50% had the highest viscosity value compared to other groups (figure 2), because the concentration of the gel base used was also the largest.

The addition of extracts can reduce the viscosity because it is liquid, so the higher the concentration of plant extracts added, the smaller the viscosity value.[9] In addition to this, it could also be due to the synergistic process, namely the release of liquid trapped in the gel so that it allows the liquid to move to the surface so that the viscosity of the preparation decreases.[15]

Temperature is inversely proportional to viscosity, the higher the temperature used, the lower the viscosity value of a preparation. Because the high temperature is able to increase the distance between the particles the force between the particles will decrease and the greater the distance can cause the viscosity of the preparation to decrease. Storage packaging that is less tight and vigorous stirring during mixing of the preparation can also cause the viscosity of the preparation to decrease.[8,16] Therefore, it is recommended to store them in an airtight container and use dark-colored bottles.

CONCLUSION

It can be concluded that the pH and viscosity test of the preparation of nanoemulgel Phaleria macrocarpa's leaves at a concentration of 30% were the most stable pH and viscosity compared to concentrations of 40% and 50%.

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