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Research Article

Optimization of Stearic Acid and Triethanolamine in The Antibacterial Cream *Staphylococcus aureus* Ethanol Extract of Papaya Seeds (*Carica papaya* L.): Factorial Design Method

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ABSTRACT

The ethanol extraction of papaya seeds provides a synergistic inhibitory effect on *Staphylococcus aureus* thorugh alkaloids, flavonoids, tannins and saponins. In this research, the ethanol extract of papaya seeds was formulated in cream preparations and optimized. The optimization of stearic acid as the oil phase and TEA as the water phase resulted in a cream with good physical properties and stability. The parameters used to observe the physical properties and stability of the ethanol extract cream of papaya seeds were organoleptic, homogeneity, pH, centrifugation, viscosity, spreadability, and adhesion. Optimization data analysis using Design Expert 12 free trial. The result of optimization is stearic acid contributed most dominantly to the response to increase viscosity by 73.739% and adhesion by 91.695%, while TEA contributed to the increased in dispersibility by 13.107%. The optimum amount of composition used for stearic acid was 5 grams and TEA was 2 grams. The research showed that inhibitory activity of papaya seed ethanol extract at a concentration of 20% was classified as moderate. However, at concentrations of 40%, 60%, 80%, and 100%, the inhibitory activity is quite strong.

INTRODUCTION

Papaya seeds (*Carica papaya* L.) are a part of papaya plants that can provide antibacterial activity. However, they often become waste and are not used. The antibacterial activity of papaya seeds has been stated by Torar (2017) to provide the antibacterial activity of *Pseudomonas aeruginosa* and *Staphylococcus aureus (S. aureus). S. aureus* is a gram-positive bacterium and is a normal flora that can be a pathogen in humans.

S. aureus is a leading cause of skin and tissue infections such as purulent cellulitis and abscesses (boils) (Tong *et al*., 2015). Therapy against *S. aureus* infection is currently increasingly difficult due to the presence of penicillin-resistant strains both in hospitals and communities (Santosaningsih *et al*., 2011). Cases of resistance are increasing and becoming a major problem throughout the world, but not accompanied by the discovery of new antibiotics (Al-Talib *et al*., 2015). Therefore, researchers want to take advantage of natural ingredients (papaya seeds) as antibacterials in the hope that they have the same benefits as synthetic materials and reduce the buildup of waste from papaya seeds by utilizing them. Phytochemical compounds that play a role are tannins, flavonoids, phenols, alkaloids, terpenoids and saponins. These are extracted by 70% of ethanol because ethanol 70% are semi-polar (Eke *et al*., 2014).

Skin conditions of patients infected with *S. aureus* such as boils, which are festering and watery, so that therapy in these patients must be maintained by humidity (Tong *et al*., 2015). There is no research into the antibacterial cream ethanol extract of papaya seeds. Based on this, the researchers formulated papaya seeds in cream preparations for antibacterial treatment

because the cream, especially type O/W is a type of cream that is able to increase percutaneous absorption so that it will provide an optimum effect and can also maintain skin moisture (Engelina, 2013).

According to Nonci (2016), one component in cream preparations that affects the physical stability of the cream is an emulgator. The combination of stearic acid and triethanolamine (TEA) will react *in situ* to produce a salt, namely triethanolamine stearate, which functions as a very stable emulgator of O/W type cream preparations (Nonci *et al*., 2016). Research conducted by Bassey (2015) states that stearic acid is more stable than linolenic acid. The optimum amount of the two ingredients will provide good physical properties and stability for the cream to use (Chomariyah *et al.,* 2019).

Based on these, both components will be optimized with the *factorial design* method because this method can determine the interaction effect of 2 factors. The responses that will be seen are the viscosity, spreadability, and adhesion responses.

METHODS

The tools used in this study include an oven (Memmert UF 260), analytic placing (Nagata), *rotary evaporator* (BUCHI Rotavapor R-300), water bath (Gerhardt), hot plate (IKA C-MAG HS 7), micropipette (SOCOREX) ®), autoclave (ALP KT-40), *Biological Safety Cabinet* (ESCO® class II type A2 series 95067), *nephelometer* (POENIX SPECREF 440910), *Rheosys* viscometer (MICRA Merlyn VR), freezer (Samsung), centrifugator (Memmert), pH meter (OHAUS® ST 10), scatter power test equipment, sticky power test equipment, and other glassware.

The materials used in this study includes dried papaya seeds obtained from papaya farmers in the Tourism Village of Pandowoharjo Hamlet, Sleman, Indonesia. Nutrient Broth (NB) media (OXOID® grade: P.A.), Nutrient Agar (NA) media (OXOID® grade: P.A.), aquadestilata, *Staphylococcus aureus* ATCC 25923 (sensitive) obtained from the Yogyakarta Laboratory of Health and Calibration Laboratory, ampicillin 1%, blank disc, DMSO 10%, FeCl3, Mayer reagent, NaOH 10 %, ethanol 70%, ethanol 95%, stearic acid, triethanolamine (TEA), and other ingredients.

Ethanol Extract of Papaya Seeds

Papaya seed simplex powder (do not use a certain powder size) was extracted by maceration. The simplicia powder weas weighed as much as 85 grams and then put into the erlenmeyer, then immersed in 95% ethanol as much as 500 mL then the erlenmeyer was covered with aluminum foil and left for 4 days while occasionally stirring. Filtering is done using filter paper so as to produce filtrate and residue. The filtered residue then goes through the process of remaceration with 95% ethanol as much as 250 mL and then covered with aluminum foil and left for 2 days while occasionally stirring, then filtered (Torar *et al*., 2017).

Filtrate one and filtrate two were mixed into one and evaporated using a *rotary evaporator*, then concentrated using a water bath to obtain a thick extract (Torar *et al*., 2017). The concentrated extract obtained was determined by fixed weight to ensure that the extract obtained was free of solvents (Depkes RI, 2014).

Phytochemical Screening

Test for Flavonoid: 1 mL of the extract is added with a few drops of 10% NaOH. The appearance of orange shows the presence of flavonoids (Ikalinus *et al*., 2015).

Test for Alkaloids: 1 mL of the extract is added with 2 drops of Mayer's reagent solution. The appearance of formation of white or yellow lumpy deposits shows the presence of alkaloids (Ikalinus *et al*., 2015).

Test for Saponin: The extract is boiled with 20 mL of water in a water bath. The filtrate is shaken and allowed to stand for 15 minutes. The formation of a stable foam showed positive samples containing saponins (Ikalinus *et al*., 2015).

Test for Tannins: The extract is boiled with 20 mL of water and then filtered. A few drops of $FeCl_3$ were added to the sample. Positive reaction if greenish-brown or black-blue color is formed which indicated tannins (Patel *et al*., 2014).

Test for Phenolic: The extract is diluted to 5 mL with distilled water. To that add a few drops of neutral 5% ferric chloride solution (Lohidas *et al*., 2015).

Antibacterial Activity Test for Papaya Seed Ethanol Extract

Testing the antibacterial activity of papaya seed ethanol extract against *S. aureus* was carried out using 6 mm diameter disc paper. Culture in NB as much as 0.2 mL was added to 15 mL NA which was already solid. Papaya seed ethanol extract with a concentration of 20%, 40%, 60%, 80%, and 100% was taken as much as 20 µL

using a micropipette and injected on sterile paper discs and then put on NA media that had been inoculated using test bacteria. Incubation was carried out at 37℃for 24 hours. The positive control used was ampicillin 1% according to CLSI (2013), in which the *S. aureus* antibacterial test (not resistant) by the disc diffusion method used the penicillin group and also penicillin group as an empirical therapy for *S. aureus* infection. The negative control used DMSO 10%. Observations were made on the formation of an irrational inhibitory zone around the disc paper (Muharni *et al*., 2017).

Papaya Seed Ethanol Extract Cream Formulation

Methods for making Creams, the formula is in Table 1 (Ekowati and Ningsih., 2014): (i) The oil phase, which is stearic acid, cetyl alcohol, liquid paraffin, olive oil and methylparaben (diluted with ethanol) is heated at 70℃ above water bath on a porcelain cup until it melts; (ii) The water phase, i.e. TEA, glycerin, propylparaben (diluted with ethanol) and aquadest is poured into a porcelain cup and heated at 70℃using a water bath; (iii) The water phase is put into the oil phase of a warm mortar and then homogenized to form a cream base.

Table 1. Papaya seed ethanol extract cream formula

Ingredients	F1	FA	FB	FAB
Papaya seed ethanol extract 20% b/v	5 mL	5 mL	5 mL	5 mL
Stearic acid (g)	5	10	5	10
Cetyl alcohol (g)	3	3	3	3
Glycerin (g)	10	10	10	10
Liquid Paraffin (g)	10	10	10	10
Olive oil (g)	10	10	10	10
TEA (g)	2	2	4	4
Methylparaben (g)	0.025	0.025	0.025	0.025
Propylparaben (g)	0.015	0.015	0.015	0.015
Aquadest add (mL)	100	100	100	100

Table 2. The results of the measurement of the irradical inhibition zone of papaya seed ethanol extract on the growth of *S. aureus* bacteria

Treatment	Inhibition Zone Diameter (mm)			Average (mm)
	Replication I	Replication II	Replication III	
Control (-) DMSO 10%	0	0	0	0
Control (+) ampicillin 1%	14	15	14	14.33
Concentration 20%	9	9	11	9.67
Concentration 40%	11	11	12	11.33
Concentration 60%	12	13	13	12.67
Concentration 80%	14	14	15	14.33
Concentration 100%	17	16	16	16.33

Table 3. Organoleptic Test Results

	F1	FA	FB	FAB
Odor	Olive oil	Olive oil	Olive oil	Olive oil
Color	Yellowish white	Yellowish white	Yellowish white	Yellowish white
Shape	Semisolid	Semisolid	Semisolid	Semisolid

Table 4. pH Test Results

	F1	FA	FB	FAB
Replication 1	8.30	7.70	8.50	8.40
Replication 2	8.30	7.70	8.50	8.40
Replication 3	8.30	7.70	8.50	8.40
$\bar{X}$ ± SD	8.30 ± 0.00	7.70 ± 0.00	8.95± 0.00	8.40 ± 0.00
CV	0%	0%	0%	0%

Table 5. Centrifugation Test Results

	F1	FA	FB	FAB
Results	Did not separate	Did not separate	Did not separate	Did not separate

Tabel 6. Viscosity Test Results

Formula	Viscosity Cycle 0 ($\bar{x}$ ± SD) (Pa.s)	Viscosity after 3 cycle ($\bar{x}$ ± SD) (Pa.s)	Change in Viscosity (%)
1	8.24 ± 0.08	8.38 ± 0.02	1.71
A	24.62 ± 0.03	24.70 ± 0.17	0.34
B	4.83 ± 0.07	4.91 ± 0.04	1.67
AB	14.27 ± 0.17	14.61 ± 0.38	2.37

Table 7. Spreadability Test Results

Formula	Spreadability Cycle 0 ($\bar{x}$ ± SD) (cm)	Spreadability after 3 cycle ($\bar{x}$ ± SD) (cm)	Change in Spreadability (%)
1	6.03 ± 0.05	6.05 ± 0.13	0.33
A	5.30 ± 0.50	5.20 ± 0.08	1.88
B	6.70 ± 0.36	6.65 ± 0.17	0.74
AB	5.70 ± 0.42	6.00 ± 0.08	5.26

Table 8. Adhesion Test Results

Formula	Adhesion cycle 0 ($\bar{x}$ ± SD) (s)	Adhesion after 3 cycle ($\bar{x}$ ± SD) (s)	Change in adhesion (%)
1	4.50 ± 0.10	4.50 ± 0.00	0.00
A	5.30 ± 0.00	5.50 ± 0.10	3.77
B	4.30 ± 0.10	4.30 ± 0.10	0.00
AB	5.40 ± 0.10	5.30 ± 0.10	1.85

Ethanol extract of papaya seeds with a concentration of 20% which has been diluted in a 5 mL volumetric flask is added little by little when the base is formed and then homogenized.

Test the Physical Properties of Papaya Seed Ethanol Extract Cream

Test the physical properties of the cream, namely the organoleptic test, homogeneity test, pH test, dispersion test, viscosity test, and adhesion test. The Organoleptic test is done by observing cream preparations visually including odor, color, and shape (Solichin *et al*., 2014). The homogeneity test is done by applying cream preparations to the slide and then covered with glass preparations and observing the cream texture (Safitri *et al*., 2014). In the pH test, 1 g of the cream is weighed and diluted with 10 mL aquadest. The calibrated pH meter is placed on the diluted cream and then the results are read on the monitor (Solichin *et al*., 2014).

The spread test is carried out by weighing 0.5 g of cream placed between two round glass plates with a diameter of 15 cm, the other glass is placed on it and left for 1 minute. The diameter of the cream that spreads (by taking the average length of the diameter from four sides) is measured, then 100 g is added as an additional load, each additional load is allowed to stand after 1 minute and the cream diameter is recorded. (Pratimasari *et al*., 2015).

The viscosity test was measured using *Rheosys* viscometer and *Rheosys Micra software* (Hidayanti *et al*., 2015). An adhesion test is done by weighing as much as 1 g of cream and then applied to a glass plate with an area of 2.5 cm^2. The plates were attached to each other and then put on a weight of 1 kg for 5 minutes, then released. A discharge load of 80 g was given for testing. Time is recorded until the two plates come off. Replication is done 3 times (Wibowo *et al*., 2017).

Stability Test for Papaya Seed Ethanol Extract Cream

The stability test of the preparation is done by centrifugation test and *freeze-thaw cycle*. Centrifugation test is done by means of the cream put in a centrifugation tube and then put into a centrifugator. Samples were centrifuged at 3750 rpm for 5 hours to observe whether or not there was separation (Rindiyantoko *et al.*, 2017). The *Freeze-thaw cycle* is carried out in each formula stored at low temperature -10℃ and temperature 40℃/75% RH, alternately for 24 hours each for 3 cycles, in which 1 cycle of *freeze-thaw* is carried out for 2 days (for 6 days). Every cycle is completed, it will observe the changing

physical properties, namely adhesion, dispersal, and viscosity (Huynh-Ba *et al.*, 2009).

Analysis of Results

Optimization in this research was carried out using factorial design methods. Data analysis of physical properties (viscosity, spreadability and adhesion) using *Design Expert 12 free trial* to get the interaction of two factors at two levels for each response through the contour plot equation. The optimal area is obtained by a superimposed contour plot.

RESULTS AND DISCUSSION

Extraction Results

Papaya seed powder was extracted using the maceration method using 95% ethanol solvent and obtained a yield of 23.33%.

Phytochemical Screening Results

Phytochemical screening of ethanol extracts of papaya in accordance with research conducted by Eke (2014) with positive results for the five compounds, namely flavonoids, alkaloids, saponins, tannins, and phenolics.

Antibacterial Test Results

The results of previous studies by Torar (2017) stated that at concentrations of 20%-60% they are classified as moderate and at a concentration of 80% are classified as strong. Whereas, the results obtained in this study were at concentrations of 20%, 40%, 60%, 80%, and 100% respectively, having an irrational inhibition zone of 9,67 mm; 11,33 mm; 12,67 mm; 14,33 mm; and 16,33 mm (Table 2). Therefore, the irradiated inhibition zone obtained in this study is included in the medium category for concentration of 20% and the strong category for concentrations of 40%, 60%, 80%, and 100% according to the Davis and Stout category. Phytochemical compounds that play a role are tannins, flavonoids, phenols, alkaloids, terpenoids and saponins.

Figure 1. Homogeneity Test Results

Evaluation of Physical Properties of Cream

Organoleptic Test

Organoleptic tests include odor, color, and shape. All four formulas have the same results presented in Table 3.

Homogeneity Test

The result is that the cream looks physically homogeneous in all formulas shown by the distribution of particles evenly on the slide (Figure 1).

pH test

pH measurement using a pH meter. The recommended pH of the topical preparation is 4.5-6 (Naibaho, 2013). The results in Table 4 show that the four cream formulas are not in the expected pH range because according to Elcistia *et al.* (2018), TEA mixed with stearic acid will form anionic soap with a pH of around 8 and form a stable and smooth type o / w emulsion. Even so, the result of pH is still acceptable for the human skin in general. Therefore, for the stability of the preparation, it can be said that the pH is suitable.

In addition, one of the ingredients that greatly influences pH to be high is TEA and if it is changed to regulate pH it will influence the optimized factor.

Centrifugation Test

Centrifugation test aims to show the shelf life of preparations for one year, which is indicated by the absence of phase separation in the preparation.

The test results are shown in Table 5, which shows that all four formulas are stable because there is no phase separation.

Viscosity Test

The expected viscosity range is 4-40 Pa.s (Genatrika *et al*., 2016). Viscosity test results are shown in Table 6. The highest viscosity value is obtained in formula A. It is influenced by the amount of stearic acid used. In formula A, stearic acid is used at a high level and TEA at a low level. Conversely, formula B has a lower viscosity value than the four formulas made. The greater the concentration of stearic acid in the formula, the higher the viscosity produced. Conversely, the lower the concentration of stearic acid, the viscosity of the cream will be lower. These results are consistent with research by Dina (2017) which states that stearic acid can increase the viscosity of preparations compared to TEA.

Spreadability Test

The theoretical value of the spreadability of semifluid cream preparations is 5-7 cm (Garg *et al*., 2002). The results of the testing of the distribution of three cycles of papaya seed ethanol extract cream can be seen in Table 7. Based on the results obtained, all formulas fall into a predetermined range, which is 5-7 cm. In Table 7, the spreadability of each formula is different. The addition of TEA concentration, in this case, the concentration of TEA at the high level of formula B and formula AB, the higher the distribution power.

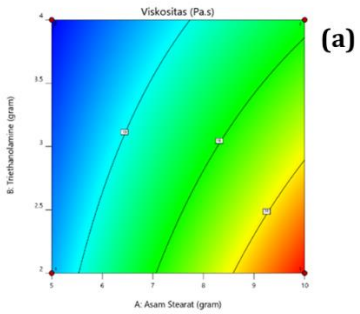
The spread of cream is related to the viscosity of the cream because of the lower the viscosity of the cream, the ability of the cream to flow higher so that the cream is able to spread easily and evenly distributed (Dina *et al*., 2017).

Adhesion Test

The expected adhesion to the cream preparation is > 4 seconds (Genatrika *et al*., 2016). Based on the results of the adhesion test found to meet the requirements (Table 8). In formula A and formula AB, it has a longer adhesion than formula 1 and formula B. This is because the formulas A and Formula AB have a higher viscosity value, where a higher viscosity value will increase the sticking time of preparation (Dina *et al*., 2017). A good cream can guarantee effective contact time with the skin so that the intended use can be achieved even if it is not too sticky when used (Dina *et al*., 2017).

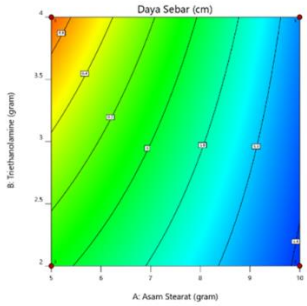
Determining the Optimal Formula for Papaya Seed Extract Cream

Cream optimization in this study is based on testing of the viscosity test, the dispersion test, and the stickiness test on the 0 cycle. The results of each test of the physical properties of the mixture of materials will obtain the physical properties profile of the *Design Experts 12 free trial*. The response of viscosity, dispersion, and adhesion is indicated by Y, X_1 is stearic acid, X_2 is TEA and X_1X_2 is the interaction between stearic acid and TEA. Based on these results, stearic acid and its interaction with TEA (on the adhesion response) has a positive value which means it has the effect of increasing the viscosity and adhesion of the preparation. TEA has a negative value on the viscosity and adhesion response, which means it has the effect of reducing the viscosity and adhesion of the preparation. These results are in accordance with the theory which states that viscosity is inversely proportional to spreadability (Dina *et al*., 2017) where the response of stearic acid spreads and their interactions has a negative value, which means stearic acid and the interaction of the two factors has an effect on reducing the spreadability of the papaya seed ethanol extract cream.



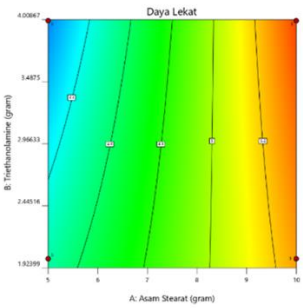
(a)

$$Y = 5{,}610 - 0{,}0367\ (X_1) + 0{,}567\ (X_2) - 0{,}050(X_1\ X_2)$$



(b)

$$Y = 5{,}610 - 0{,}0367\ (X_1) + 0{,}567\ (X_2) - 0{,}050(X_1\ X_2)$$



(c)

$$Y = 4{,}233 + 0{,}093\ (X_1) - 0{,}250\ (X_2) + 0{,}030\ (X_1X_2)$$

Figure 2. Contour plots of viscosity (a), spreadability (b), and adhesion (c)

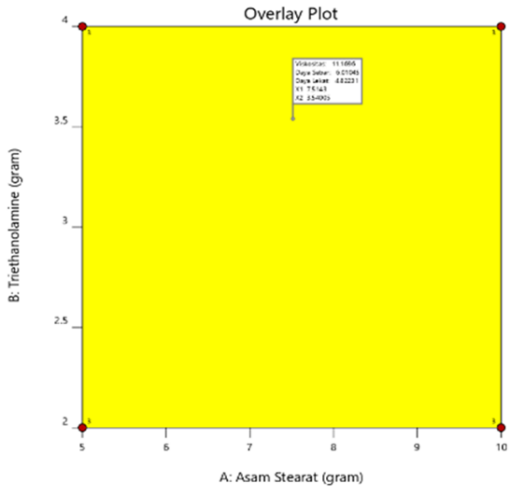


Figure 3. Contour Plot Superimposed from the response of viscosity, spreadability, and adhesion

The reverse event in TEA has a positive value, which means TEA has the effect of increasing spreadability. Both factors and their interactions have a significant effect on the response to viscosity, dispersion, and adhesion with a *p-value* <0,0001.

Increases or decreases in stearic acid and TEA against tests of viscosity, dispersion, and adhesion are shown in Figures 2. from blue (low) to orange (high). The optimum composition area after the viscosity, dispersion, and adhesion response are plotted in one graph shown in Figure 3.

The optimum composition area is indicated by the yellow area, while the gray area is the area that is not included in the optimum composition but is not included in the results of this analysis. The range of viscosity, spreadability, and adhesion used in determining the optimum composition area are 4-40 Pa.s, 5-7 cm and >4 seconds. Based on the results of the Superimposed Contour Plot, the optimum formula point of the papaya seed extract cream is determined, namely 5 grams stearic acid and 2 grams TEA. The choice of the point is based on consideration, that all formulas are included in the optimum formula, which means that papaya seed ethanol extract in cream preparations can be formulated in stearic acid and high or low-level TEA. Therefore, a formula with low-level stearic acid and high-level TEA was chosen so that the ingredients used were more efficient while still providing a stable cream preparation.

CONCLUSION

Papaya seed ethanol extract has antibacterial activity against *S. aureus* at a moderate concentration of 20% and strong at a concentration of 40%, 60%, 80%. The optimum amount of stearic acid in this formulation is 5 grams and TEA is 2 grams, which provides good physical properties and stability for preparations in ethanol extracts of papaya seeds. Stearic acid will have the dominant effect of increasing the viscosity and adhesion but decreasing the dispersibility with the increase in quantity, while TEA has the effect of decreasing the viscosity and adhesion but increasing the dispersibility with the increase in number. Suggestions for further research are to do antibacterial testing in this cream preparation.

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