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OPTIMATION OF SORBITOL, GLYCERINE, AND XANTHAN GUM COMBINATION IN MUCOLITIC SYRUP OF *Hibiscus rosa-sinensis* LEAVES EXTRACT USING MIXTURE DESIGN (D-OPTIMAL)

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ABSTRACT

Ethanolic extract of *Hibiscus rosa-sinensis* L leaves has in vitro mucolytic activity which at 0.75% has in vitro mucolytic activity equivalent to 0.1% acetylcysteine. However, the extract has a bitter taste and low acceptability. Therefore, Hibiscus leaf extract is formulated in the form of syrup by optimizing the excipient. In this research, the optimized excipients were sorbitol, glycerin, and xanthan gum since those excipients have roles to improve the acceptability of syrup by using the Mixture Design (D-Optimal) method. The properties of physical syrup that were evaluated included acidity (pH), viscosity, ease of pouring, and taste. The difference between the optimum formula prediction from software and the physical characteristics syrup experimental results were continued using a one-sample of t-test with a confidence level of 95%. The mucolytic effectiveness of optimal syrup was tested and compared to the positive control (0.1% acetylcysteine syrup). The D-Optimal Mixture Design had a desirability value of 0.84, with the optimal percentage of excipients being 34.57% of sorbitol solution, 15.53% of glycerin, and xanthan gum with 0.10%. The results pH, viscosity, ease of pouring, and taste of optimum syrup showed no significant difference (sig > 0.05) between the experimental results and the predictions *Design Expert® software* version 7.1.5. It is confirmed the validation of the software used for the experiment. The optimum syrup of ethanolic extract of Hibiscus rosa-sinensis L. leaves has mucolytic activity equivalent to acetylcysteine syrup at 0.1%.

Keywords: *D-Optimal Design Mixture Design,* Excipients, *Hibiscus rosa-sinensis* L Leaves Extract, Mucolytics, Optimation.

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INTRODUCTION

The use of natural ingredients used for treatment is increasing due to the level of safety and lack of side effects. Indonesia is a tropical country that has a lot of biodiversities that can be used as a source of natural medicinal ingredients.[1] Among various kinds of plants, Hibiscus (*Hibiscus rosa-sinensis* L) is a plant that has the potential as a medicine. Hibiscus leaves are efficacious in softening the skin and shedding phlegm, and are antipyretic.[2] The ethanolic extract of Hibiscus leaves at a concentration of 0.75% had an in vitro mucolytic activity equivalent to 0.1% acetylcysteine. The extract contains several compounds including saponins, flavonoids, polyphenols, and triterpenoids.[3] However, Hibiscus leaf extract has a bitter taste and low acceptability. Therefore, to improve aesthetics and optimize its utilization, Hibiscus leaf extract is formulated in the form of syrup. Components added to improve the aesthetics and acceptability of the syrup include sweeteners, viscosity enhancers, stabilizers, and cosolvents. The sweetener used is sorbitol, in the formula in addition to improving the taste of syrup preparations, maintaining syrup stability from cap locking.[4] Sorbitol also serves to cover the bitter taste of the ethanolic extract of Hibiscus leaves so that the

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syrup preparation is expected to be more acceptable. Sorbitol is a type of sweetener that is free of sugar, therefore this syrup preparation is quite safe for consumption by diabetics.[5] Xanthan gum in syrup is used to increase viscosity which improves the aesthetics of the syrup used. Besides, it adds more sensory quality (flavour release, mouth feel) to the final product.[6] Glycerin added to the syrup acts as a cosolvent to help dissolve the extract of Hibiscus leaves so that it is easier to be formulated in syrup dosage form.[7] An experimental design statistical application used to optimize the amount of each component added is Response Surface Methodology (RSM) such as D-Optimal Design. The software is used to determine the effect of each component and the effect of the combination of these components on the physical properties of the syrup.[8] The optimal formula obtained was tested for mucolytic activity based on the decrease in the viscosity value of cow mucus compared to the decrease in viscosity that occurs with the addition of 0.1% acetylcysteine syrup as positive control.[9] This study was purposed to obtain the optimum formula for the syrup preparation of Hibiscus rosa-sinensis (*Hibiscus rosa-sinensis* L.) leaf extract according to the physical properties using the D-Optimal Mixture Design method and to determine the mucolytic activity of the optimum formula for the ethanolic extract of the hibiscus leaf extract.

EXPERIMENTAL

Materials

Hibiscus leaf (*Hibiscus rosa-sinensis* L.), distilled water, aluminium foil, gallic acid, tartaric acid (pharmaceutical grade), 0.1% acetylcysteine, 70% ethanol (technical grade), ethanol (pro analysis grade), glycerin (pharmaceutical grade), flannel cloth, filter paper, methylparaben (pharmaceutical grade), $AlCl_3$, respiratory mucus from cow, Na_2CO_3, NaOH, Glassware (Pyrex®), sieve number 12, Chopper Machine (Miyako®), Buncher funnel (Pyrex®), glass, fan, electric stove, micropipette, digital balance (Ohauss®), oven (Memmert®), pH meter (Hanna Instruments®), pycnometer (Pyrex®), volume pipette, UV-VIS spectrophotometry (Merck®), stand and clamp, stopwatch, vacuum, Ostwald viscometer (Pyrex®), aluminium pan and water bath.

Extract Preparation

Harvested hibiscus leaves were cleaned, dried at 60^0 C and powdered. One part of simplicia was macerated in 10 parts of petroleum ether. Every 24 hours for 3 days, petroleum ether was replaced. This process was purposed to delipidate hibiscus leaves. A delipidated ethanol extract of Hibiscus leaves was dried and macerated in 70% ethanol for three days, with the same volume of solvent refilled every 24 hours until a clear solvent was achieved.[10] The filtrate was evaporated in rotary evaporator at 50°C and thick extract obtained was stored in desiccator.[11,12]

Syrup Formulation

Mucolytic syrup formula was created in 17 different formulations with three replications (triplo). Each mucolytic syrup formula contains propylparaben, methylparaben, xanthan gum, tartaric acid, strawberry flavour, red food colouring, delipidated ethanol extract of Hibiscus leaves, and distilled water. Mixture Design (D-Optimal) of Design Expert Version 7.1.5 was used to create and optimize the formula. Various ratios of three excipients were optimized: glycerin, xanthan gum, and sorbitol (Table-1). The Mucolytic syrup formula was created by carefully weighing all of the ingredients. Distilled water was heated to 50^o degrees Celsius before adding propyl and methyl parabens and stirring until completely dissolved. The xanthan gum was then slowly added while stirring for 2 minutes and allowed to cool at room temperature. Tartaric acid was dissolved in a small amount of water before being added to the mixture. The thick extract of Hibiscus leaves was dissolved in a small amount of distilled water, then glycerin was added and stirred until homogeneous, and cooled xanthan gum was added to the solution. Sorbitol, flavouring, and colouring food were added while gently stirring. The volume is then increased to the limit (100 ml) with distilled water and stirred again until homogeneous.

Analysis of Syrup Response Variables

pH Value

Digital pH meter that functioned on the glass electrode concept was used to measure the pH.[13] Before use, the digital pH meter was first calibrated. After immersing the pH meter in the sample, the pH readings displayed on it were recorded until a stable value was discovered.[14]

Syrup Viscosity

Viscosity of syrup can be determined by using Ostwald viscometer.[15] First, clean the Ostwald viscometer thoroughly with warm chromic acid or acetone. The viscometer was filled with the tested syrup and allowed to drain through the capillary. The boundary markers of capillary were two: markings of lower and top border. The stopwatch would switch "on" when the syrup flowed and reached the upper limit boundary and "off" when the flow reached the lower limit barrier. The time it took for the syrup to flow between the two markings was then recorded. The viscosity measurement value obtained with the Ostwald viscometer was computed using the formula published by Liu *et al.*[16]

Taste

The taste response test was designed to assess the patient's acceptance of Delipidated ethanol extract of Hibiscus leaves syrup, specifically the taste and aesthetics of the syrup. The experiment was carried out by giving syrup to 20 participants. Respondents would complete a questionnaire assessing the syrup's taste, odour, and aesthetics.[17]

Pourability Test

The goal of this test was to show how easily the syrup could be poured so that it could be administered to the patient. Pouring 25 mL of syrup at a 45-degree slope and recording the time it took for the syrup to pour completely from the container was used for the ease of test pouring.

Mixture Design (D-Optimal)

Design Expert® software version 7.1.5 was used to optimize the Hibiscus leaf ethanolic extract syrup using the D-Optimal Mixture Design approach. Where there are 17 runs (Table-2); three independent variables, namely glycerin, sorbitol, and xanthan gum; in two levels, low and high. The data used to optimize the ethanolic extract syrup preparation, which is the dependent variable, are the physical properties of the syrup preparation, which include pH, pourability, viscosity, and taste responsiveness (Table-1). Glycerin, sorbitol, and xanthan gum made up 50.2% of the total. Table-1 shows the goal and importance values for each component are set at the same level, indicating that the three components play an equal role in optimizing syrup preparations. The goal value and importance on the dependent variable, namely pouring power, pH, and viscosity, are also set at the same level because these three variables are physical properties that are equally important in syrup optimation.

Table-1: Causal Factors and Response Variables in D-Optimal Mixture Design to Determine the Optimum Formula

Variables	Constraints			
Causal factors	Low level (%)	High level (%)	Goal	Importance
Gliserin	15.05	30.00	*In range*	3
Sorbitol	20.00	35.00	*In range*	3
Xanthan gum	0.1	0.25	*In range*	3
Response	Low limit	Upper limit	Goal	Importance
Pourability	0.86	1.27	*In range*	3
pH	2.65	2.79	*In range*	3
Taste	2.85	3.60	*maximize*	3
Viscosity	2.02	5.66	*In range*	3

The goal value in the taste response test is to maximize because taste responsiveness is a physical trait that is directly related to the acceptability of syrup preparations. The greater the taste response value, the more acceptable the syrup preparation. The importance level is three. Viscosity is an equally important physical characteristic in the optimization of syrup preparations due to its responsiveness to taste, pourability, and pH. Pourability, pH, viscosity, and taste responsiveness are physical properties used to optimize the syrup preparations of the ethanolic extract of *Hibiscus rosa sinensis* L. leaves to obtain the optimal syrup.

Table-2: The Compositions of Causal Factors and Physical Characteristics (Response Variables) of Model Formulations.

Formula	Run order	Causal factors (%)			Response variables			
		Glycerin	Sorbitol	Xanthan gum	Physical characteristics			
					pH	Viscosity	Taste	Pourability
1	7	15.10	35.00	0.100	2.76	3.26	3.6	0.88
2	10	22.500	27.450	0.250	2.68	5.67	3.3	1.02
3	11	29.950	20.000	0.250	2.65	5.63	3.05	1.24
4	12	15.067	34.967	0.167	2.70	4.57	3.5	0.86
5	3	22.550	27.550	0.100	2.72	4.19	3.25	1.04
6	17	15.050	34.900	0.250	2.68	5.53	3.45	0.87
7	5	30.000	20.100	0.100	2.75	4.12	3.25	1.24
8	2	18.813	31.250	0.138	2.72	4.41	3.3	0.91
9	1	26.262	23.800	0.138	2.73	4.53	3.15	1.16
10	6	20.017	29.967	0.217	2.65	4.71	3.2	0.96
11	16	25.033	25.033	0.133	2.70	4.26	3.15	1.14
12	15	25.000	24.967	0.233	2.69	4.71	3.25	1.11
13	13	15.100	35.000	0.100	2.79	2.03	3.55	0.87
14	4	22.550	27.550	0.100	2.76	3.96	3.35	1.01
15	14	29.950	20.000	0.250	2.65	5.10	3.15	1.28
16	9	30.000	20.100	0.100	2.75	4.25	2.85	1.26
17	8	15.050	34.900	0.250	2.68	5.17	3.4	0.88

Fitting the Model

Analysis of variance (ANOVA) was used to examine the experimental data in Table-2, and numerous terms, such as the sequential model sum of squares, lack-of-fit tests, and model summary statistics, were employed to fit the models.[18]

Table-3: ANOVA Statistics Showing the Effect of the Formulation Parameters on the pH, Pourability, Taste, and Viscosity of Mucolytic Syrup of Hibiscus Leaves (*Hibiscus rosa-sinensis*)

pH (Acidity)					
Source	Sum of Squares	df	Mean square	F value	p-value Prob > F
Model (Linear Mixture)	0.024	2	0,012	29.65	< 0.0001
Residual	5.595E-003	14	3.996E-004		
Pure Error	1.250E-003	5	2.500E-004		
Lack of Fit	4.345E-003	9	4.828E-004	1.93	0.2426
Pourability					
Source	Sum of Squares	df	Mean Square	F value	p-value Prob > F
Model (Linear mixture)	0.37	2	0.18	182.33	< 0.0001
Residual	0.014	14	1.010E-003		
Pure Error	3.772E-003	5	7.544E-004		
Lack of Fit	0.010	9	1.153E-003	1.53	0.3337
Taste					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model (Linear mixture)	0.42	2	0.21	19.54	< 0.0001
Residual	0.15	14	0.011		
Pure Error	0.092	5	0.018		
Lack of Fit	0.058	9	6.432E-003	0.35	0.9199
Viscosity					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model (Linear mixture)	9.18	2	4.59	17.17	< 0.0001
Residual	3.74	14	0.27		
Pure Error	1.01	5	0.20		
Lack of Fit	2.74	9	0.30	1.51	0.3386

The coefficients of the optimized models are shown in Table-3. All of the models chosen (Linear mixture)

were statistically significant, with "p-value probability > F-value" values of 0.0001 for all responses. Furthermore, the lack-of-fit F-value used to demonstrate the model's inability to represent data was not significant ($p > 0.05$).[19] These findings suggested that the proposed models were capable of predicting responses with 95% confidence.[15,16]

In-vitro Mucolytic Activity Testing of the Optimized Syrup

Mucolytic activity was determined based on the ability of the optimal syrup of Hibiscus leaf extract to reduce the viscosity of a 20% mucus-buffer solution at 37°C. This was compared to a positive control of 0.1% acetylcysteine and a negative control of optimal syrup without extract. Formula was used to calculate viscosity from the flow time and density of the test solution:[20]

$$\eta \text{ test solution } 37°C = \frac{\rho \text{ test solution } 37°C \times t \text{ test solution } 37°C}{\rho \text{ aquadest } 37°C \times t \text{ aquadest } 37°C}$$

t = the amount of time required for the sample to flow in seconds
ρ = density (g/mL).

Analysis of Mucolytic Activity

The data obtained from the syrups in vitro mucolytic activity were analyzed using ANOVA and one sample t-test with a 95% confidence level.

RESULTS AND DISCUSSION

Effect of Glycerin, Sorbitol, and Xanthan Gum on Syrup pH

The equation for the pH response based on the Coutour plot is:

$$R_1 = 2.74\ (A) + 2.76\ (B) - 5.80(C)$$

Where, R_1= pH ; A = glycerin; B = sorbitol; C = Xanthan Gum

The equation shows that sorbitol and glycerin have positive values, indicating that sorbitol and glycerin can raise the pH of syrup preparations. Because glycerin has a higher coefficient than sorbitol, it can be said that glycerin has nearly the same effect on increasing the pH of the syrup. Xanthan gum has a negative value which means that xanthan gum can lower the pH of syrup preparations. The effect of Xanthan gum in lowering the pH is greater than the effect of sorbitol and glycerin in increasing the pH. This is because the value of xanthan gum is greater than that of sorbitol and glycerin. This can also be seen in the contour plot of pH (Fig.-1), where the greater the concentration of xanthan gum, the lower the pH of the syrup. The colour of the area is shown in the contour plot image, which progresses from yellow to green to blue. The colour indicates that the pH of the preparation ranges from highest to lowest.

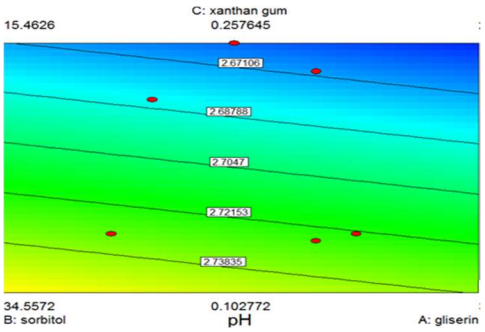

Fig.-1: Contour Plot of pH

Effect of Glycerin, Sorbitol, and Xanthan Gum on Syrup Pourability

The equation for the pourability response based on the contour plot is:

$$R_4 = 1.24\ (A) + 0.85\ (B) + 2.62\ (C)$$

Where, R_4= pourability; A = glycerin; B = sorbitol; C = Xanthan Gum

The equation shows that glycerin, sorbitol, and xanthan gum all have positive values, indicating that the three ingredients can all increase pourability. When compared to sorbitol and glycerin, Xanthan gum has the greatest effect on increasing pourability. Increasing pourability was not followed by an increase in xanthan gum in the pourability response contour plot (Fig.-2). This means that the ability of xanthan gum to increase pourability is not proportional to its concentration.

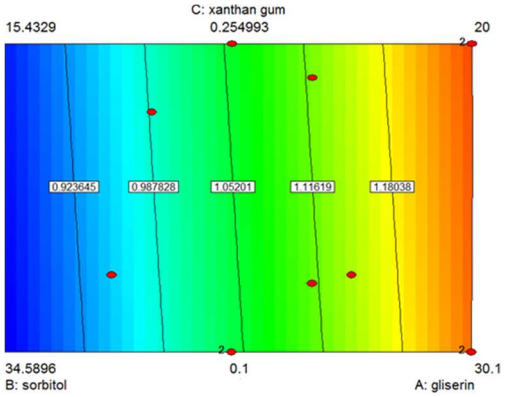

Fig.-2: Coutour Plot of Pourability

Effect of Glycerin, Sorbitol, and Xanthan Gum on Syrup Taste

The equation for the syrup taste based on the contour plot is:

$$R_3 = 3.14\ (A) + 3.46\ (B) + 0.23\ (C)$$

Where, R3= syrup taste; A = glycerin; B = sorbitol; C = Xanthan Gum

The equation shows that glycerin, sorbitol, and xanthan gum all have positive values, indicating that the three ingredients can each increase taste response. Sorbitol has a higher coefficient (3.46) than glycerin (3.14). This means that sorbitol has a greater effect than glycerin on increasing the taste response of syrup. Xanthan gum has a coefficient of 0.23. This means that xanthan can improve syrup taste responsiveness, but the coefficient value is too small, so the effect of xanthan gum on syrup taste responsiveness is minimal or can be ignored. This is also evident in the taste response contour plot (Fig.-3), where the concentration of sorbitol increases the taste response.

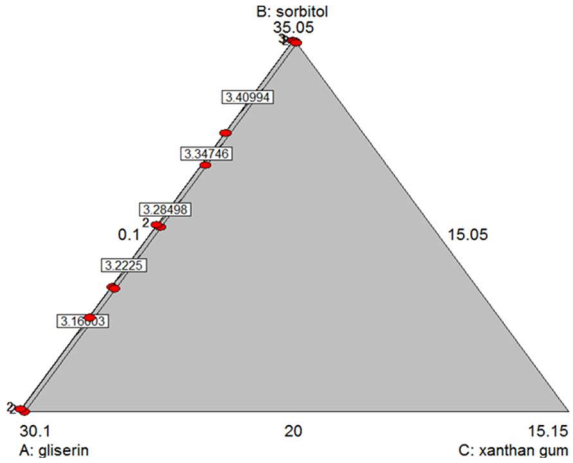

Fig.-3: Contour Plot of Syrup Taste

Effect of Glycerin, Sorbitol, and Xanthan Gum on Syrup Viscosity

Glycerin, sorbitol, and xanthan gum all have positive values in the equation, indicating that these three ingredients can increase the viscosity of syrup preparations. When compared to glycerin and sorbitol, xanthan gum has a much higher impact on raising the viscosity of syrup. The effect of xanthan gum

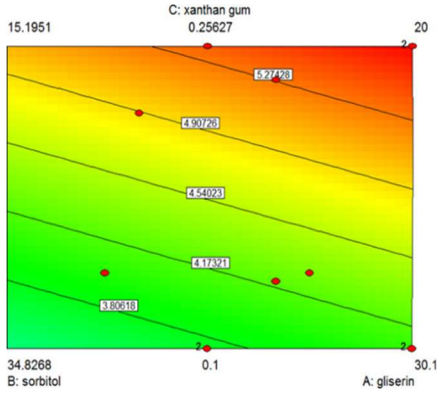

Fig.-4: Contour Plot of Syrup Viscosity

on viscosity is visible in the viscosity contour plot (Fig.-4), where the increase in viscosity is proportional to the concentration of xanthan gum. Xanthan gum is a hydrophilic biopolymer that is soluble in both cold and hot water. It has a high viscosity in low concentrations when compared to other hydrocolloid polysaccharides such as CMC, guar gum, and alginate.

The equation for the syrup viscosity based on the contour plot is:

$$R_2 = 4.06\,(A) + 3.43\,(B) + 165.92\,(C)$$

Where, R_2= viscosity; A = glycerin; B = sorbitol; C = Xanthan Gum

Optimum Formula Prediction

D-Optimal mixture Design from Design Expert® software version 7.1.5 was used to predict the optimum formula for the syrup of ethanolic extract of Hibiscus leaves. The highest desirability value, 0.840, was obtained from the software analysis. The highest desirability value is one (1). The closer the desirability value is to one (1), the better.

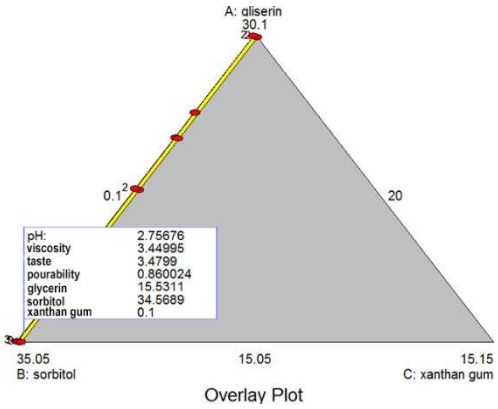

Fig.-5: Superimposed Contour Plots for pH Response, Viscosity, Taste Response, and Syrup Pourability

The optimal superimposed formula composition for a desirability value of 0.840 is glycerin up to 15.5311%, sorbitol up to 34.5689%, and xanthan gum up to 0.1%. The optimal formula's composition predicts a pH of 2.76, a viscosity of 3.45 cPs, a taste response of 3.48, and a pouring power of 0.86 seconds (Fig.-5).

Optimum Formula Prediction and Experiment Confirmation

The response predictions generated by the D-Optimal Mixture Design software are then compared to the experimental results. One Sample T-Test was used for statistical analysis and to test the significance of the difference between the average experimental results and the software's predicted value. The pH response, viscosity, taste response, and pourability showed no significant difference between the D-Optimal Mixture software predictions and the experimental results. This can be seen from the Sig-2-tailed value of each response which is more than 0.05 (Table-4).

Table-4: Analysis of Variance (ANOVA) Verification of Viscosity Response Optimization Results

Response	Prediction	Experiment	*Sig-2 tailed*	Conclusion
pH	2.757	2.78 ± 0.01	0.057	Not significant
Viscosity	3.449	3.502 ± 0.038	0.145	Not significant
Taste	3.479	3.45 ± 0.510	0.796	Not significant
Pourability	0.859	0.853 ± 0.120	0.926	Not significant

Mucolytic Effect of Optimum Syrup Formula

The lower the relative viscosity and percentage, the greater the mucolytic effect. The relative percentage is the ratio of the test solution's viscosity to that of the negative control. Table-5 shows that the positive control, acetylcysteine, has the greatest mucolytic power, followed by syrup from the optimum formula. The optimal extract-free formula had a higher relative viscosity and percentage than the negative control. This is because syrup without extract can increase viscosity and also lacks mucolytic compounds.

Table-5: Viscosity of Positive Control, Negative Control, Optimum Formula Syrup, and Optimum Formula Syrup Without Extract

Test Solution	Viscosity (Cps)	Relative Percentage
Positive Control	1.555 ± 0.000	37.42 %
Negative Control	4.155 ± 0.407	100 %
Optimum Formula	1.627 ± 0.135	39.16 %
Optimum Formula without extract	4.270 ± 0.195	102.75 %

The statistical analysis was continued with the Tukey-HSD test to see if there was a significant difference between each test solution, and the results are shown in Table-6. A one-way ANOVA test was performed to determine the difference in mucolytic power between each solution, yielding a sig value of 0.000, which was less than 0.05. As a result, it is possible to conclude that the test solution differs significantly.

Table-6: Tukey-HSD Test

(I) Solution	(J) Solution	Sig.
Optimum Formula	Negative Control	0.000
	Positive Control	0.981
	Mucus 20%	0.000
Negative Control	Optimum Formula	0.000
	Positive Control	0.000
	Mucus 20%	0.930
Positive Control	Optimum Formula	0.981
	Negative Control	0.000
	Mukus 20 %	0.000
Mucus 20%	Optimum Formula	0.000
	Negative Control	0.930
	Positive Control	0.000

*. The mean difference is significant at the 0.05 level.

According to Table.6, the optimum formula has mucolytic power that is not statistically different from the positive control (0.1% acetylcysteine syrup), as the sig value between the two is 0.981 > 0.05. This implies that the acetylcysteine syrup dose used is equivalent to the optimum syrup of the ethanolic extract of Hibiscus leaves in terms of reducing mucus viscosity in vitro.

CONCLUSION

The optimum formula was obtained using Design Expert® software on D-Optimal Mixture Design version 7.1.5, with glycerin up to 15.53%, sorbitol up to 34.57%, and xanthan gum up to 0.1%. The optimum formula obtained in the experiment had a viscosity response, ease of pouring, acidity, and taste response that did not differ significantly from the response prediction provided by Design Expert® software version 7.1.5. In vitro mucolytic activity of optimum formula of Syrup from ethanolic extract *Hibiscus rosa-sinensis* L leaves is equivalent to 0.1% acetylcysteine syrup.

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CONFLICT OF INTERESTS

The authors state that they have no known conflicting financial or personal interests that may have seemed to affect the work presented in this study.

AUTHOR CONTRIBUTIONS

All of the authors made major contributions to this work, engaged in review/editing, and approved the final text for publication. The writers' research profiles may be confirmed using their ORCID ids, which are shown below:

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