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Optimization of Gelatin and Pectin Combination as a Gelling Agent in the Formulation of Flawless Jelly Preparations with Ethanol Extracts of Meniran *(Phyllanthus Niruri)* and Kelor *(Moringa Oleifera)*

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ABSTRACT

Formulating the Jelly Flawless preparation using extracts of meniran (*Phyllanthus niruri*) and Moringa (*Moringa oleifera)* which are rich in antioxidants. Antioxidants have an important role in the body by protecting cells from damage caused by free radicals. The Jelly Flawless formulation with ethanolic extracts of meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera),* combined with gelatin and pectin, affects the hardness test, showing no significant hardness in the fourth formulation. It contains positive secondary metabolites such as tannins, alkaloids, saponins, flavonoids, quinones, and steroids. The antioxidant test results for the extracts of meniran *(Phyllanthus niruri*) and kelor (*Moringa oleifera*) were 5.55 µg/mL, higher than the positive control of vitamin C of 2.78 µg/mL, indicating that the ethanolic extracts of *Phyllanthus niruri and Moringa oleifera* contain strong antioxidants. Therefore, the Jelly Flawless formulation of ethanolic extracts of meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera*) can be produced and consumed because it contains strong antioxidants that have an important role in the body.

KEYWORDS: Meniran (Phyllanthus niruri), Kelor (Moringa oleifera), Antioksidan DPPH (2,2-diphenyl-1-picrylhydrazyl), Jelly Flawless

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I. INTRODUCTION

Free radicals are molecules with unpaired electrons, which can donate or accept electrons from other molecules. With the rapid development of time, unhealthy, irregular, and poorly controlled lifestyles such as the consumption of fast food (junk food) without regular exercise, smoking, and air pollution from vehicle exhaust and cigarette smoke, there has been a significant increase in free radicals that exceed the threshold capacity, potentially causing cell damage in the body. In the human body, free radicals can be neutralized through antioxidant defense mechanisms by binding to free radicals and highly reactive molecules without becoming free radicals themselves.

Natural antioxidants can be obtained from natural ingredients such as green and yellow leafy vegetables, and fruits. Natural antioxidants are considered safer compared to synthetic antioxidants. One of the herbal plants that contains a lot of antioxidants is meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera*).

Meniran (*Phyllanthus niruri*) is a tropical plant distributed worldwide and recognized for its use in traditional medicine as an immunomodulator, antiviral, antibacterial, diuretic, anti-hyperglycemic, and hepatoprotector. All parts of the meniran plant contain phytochemicals such as flavonoids and phenolic compounds that provide antioxidant activity. The kelor (*Moringa oleifera*) plant has high antioxidant activity with active compounds that are beneficial for health. The test results show that the secondary metabolite compounds in kelor leaves include flavonoids, tannins, saponins, and terpenoids or steroids. One of the active compounds in kelor leaves is flavonoids. Further research indicates that kelor leaves contain high levels of antioxidants and antimicrobial properties, which are due to the presence of ascorbic acid, flavonoids, phenolics, and carotenoids. Medicinal plants are generally made into herbal medicine products such as

traditional herbal drinks, instant herbal drinks, capsules, tablets, syrups, or suspensions, but not many have been processed into appealing food preparations. This processing aims to enhance the appeal of using herbal preparations. One of the interesting preparations is Jelly Flawless, which has a chewy texture and a sweet taste, so it is hoped that the production of Jelly Flawless preparations with extracts of meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera*) consumed orally, will be more favored by both children and adults.

The objective of this research is to determine the formulation of Jelly Flawless preparations combining meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera*) that meet the requirements for good physical quality evaluation, as well as to ascertain the antioxidant effectiveness of the pure extract from the combination of meniran and kelor as seen from the IC_{50} (*Inhibition Concentration 50%)* value. The results of the formulation are expected to produce a Jelly Flawless product that can help increase antioxidant intake in the body, neutralize free radicals, prevent oxidative stress, protect cells in the body, support the immune system, prevent premature aging, and aid in body detoxification.

II. MATERIALS AND METHODS

A. MATERIALS

The raw materials of meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera*) plants were obtained from Banyudono, Boyolali, Central Java. The parts of the plants used were the whole herb of meniran and kelor, which were determined at the Biology Laboratory, Faculty of Education, Muhammadiyah University of Surakarta. All other chemicals used were of analytical grade. Determination Letter No: 021/AE-1/LAB. BIO/VIII/2023

B. METHODS

Jelly Preparation

The preparation of the jelly formulation refers to previous research by Firdaus (2014) with modifications to the type of base used. The preparation is carried out as follows: gelatin is dissolved in warm distilled water at a temperature of approximately ± 40°C, and pectin is dissolved in distilled water at room temperature with a gelatin or pectin to water ratio of 1:2. The mixture is left for approximately 10 minutes until the gelatin and pectin expand and form a gel. Then, the ethanolic extract combination of meniran (*Phyllanthus niruri*) and kelor *(Moringa oleifera*) is dissolved into propylene glycol (solution A). Sorbitol, citric acid, and potassium sorbate are dissolved into a simple syrup (solution B). Afterward, solution A and B are mixed and stirred until homogeneous (solution C). Gelatin and pectin are heated in a water bath in an evaporating dish at a temperature of ± 40–50°C for 5 minutes until the gel melts. Solution C is poured into the evaporating dish, and finally, strawberry flavor and coloring are added while stirring until homogeneous. The formula is removed from the water bath and poured into jelly molds, each mold weighing approximately 2 g. The formula is left for 2 x 24 hours at room temperature (15–30°C) until the formula solidifies and can be removed from the molds. The formulation of the Flawless Jelly with meniran and kelor extracts is shown in the following table:

Table 1. Formula sediaan Jelly Flawless esktrak meniran dan kelor

Material	**Formula%**				
	F1	**F2**	**F3**	**F4**	**F5**
Meniran	15	15	15	15	15
Kelor	15	15	15	15	15
Gelatin	20	15	10	5	0
Pectin	0	5	10	15	20
Propylene Glycol	10	10	10	10	10
Sirup Simplex	25	25	25	25	25
Sorbitol	0.0075	0.0075	0.0075	0.0075	0.0075
Citric acid	0.3	0.3	0.3	0.3	0.3
Potassim Sorbat	0.1	0.1	0.1	0.1	0.1
Strawberry Seasoning	0.55	0.55	0.55	0.55	0.55
Food coloring	0.31	0.31	0.31	0.31	0.31
Aquadest (ad)	100	100	100	100	100
Total	**186.3**	**186.3**	**186.3**	**186.3**	**186.3**

Evaluation of Jelly Preparations

Organoleptis and pH Test

Organoleptis evaluation is carried out by visually observing jelly including color, shape, surface texture, aroma and overall taste of jelly. The pH determination test was carried out by dipping a pH meter into the gel mass of the jelly preparation shortly before the formula was lifted from the water bath and poured into the mold. The pH value of the preparation is measured by looking at the pH value listed on the pH meter.

Weight Uniformity Test

The evaluation of the uniformity of this weight was carried out by weighing as many as 20 jelly, then weighed one by one with a digital balance. After that, the average value, CV, Standard Deviation (SD) and the percentage deviation of the weight are calculated. The requirements of this evaluation must be in accordance with the weight uniformity table as stated in the table of weight uniformity requirements of the Indonesian Pharmacopoeia VI (2020).

Moisture Content Test

The evaluation of moisture content was carried out by heating the cup in a 100°C oven for ± 1 hour and cooling it in a desiccant for 20-30 minutes, then the cup was weighed (W0). After that put 5 g of jelly in a cup and weighed (W1). The cup is then reheated in a 100°C oven for ± 1 hour with the lid open for 3 hours. The dish is cooled again in a desiccant for 30 minutes and weighed. Reheating is carried out for 1 hour and repeated until the weight change between heating for 1 hour has an interval of ≤ 2 mg (W2). The results obtained compared to the Pharmacopoeia Indonesian edition VI (2020) are that the moisture content is no more than 35%.

Texture Profile Analysis (TPA)

Weight Uniformity Test The texture characteristics of the preparation were evaluated using the TA Plus Texture Analyzer texture analysis model. One gummy was taken from each formula at random and then measured using texture analysis. Measurements were made using a variety of probes for texture profile analysis, which included hardness and elasticity, as well as product softness. The measurement results will be obtained in the form of numerical values with the unit of hardness being the Kg force, (e.g. distance mm), and elasticity(N). Reviewed through organoleptic tests (hedonistic tests: product preference)

Phytochemical Test

After obtaining thick extracts (meniran and kelor), then phytochemical screening tests were carried out in the form of flavonoid tests, tannin tests, saponin tests, alkaloid tests, steroid tests and triterpenoids (Marisi Tambunan *et al.*, 2019).

Flavonoid Test

The flavonoid examination was carried out by means that the extract from the maceration of the sample was taken with a spatula, then a spatula of magnesium powder (Mg) and four drops of HCl 2% were added. The presence of flavonoids will be indicated by the change in the color of the filtrate to orange-red.

Tannin Test

The combination of thick extracts of meniran and kelor was added with acetone, then placed into a test tube and 1-2 drops of 1% ferric chloride reagent are added. The presence of tannins will be indicated by a change in the color of the filtrate to green or dark blue.

Saponin Test

Thick extracts (meniran and kelor) are added with acetone, then put into a test tube, then added hot water, cooled, then shaken for 10 seconds. After that, the changes that occurred were observed. Next, 1 drop of HCl 2N was added again and the changes that occurred were observed again. Positive results if foam appears stable for 10 minutes.

Alkaloid Test

The viscous extract to be examined is put into a test tube, then a few drops of HCl 2N and distilled water are added, after which it is heated over a water bath for 2 minutes, then cooled and filtered. The filtrates used for alkaloid tests are as follows:

a. Three drops of filtrate were added with 2 drops of Mayer's reagent solution, then observations were made.
b. Three drops of filtrate were added with 2 drops of Bouchardat reagent solution, then observations were made.
c. Three drops of filtrate were added with 2 drops of Wagner's reagent solution, then observations were made.

The alkaloid is positive if there is a precipitate or turbidity in at least two of the three above trials. The characteristic of the positive reaction of alkaloids is the formation of brownish-yellow color with Wagner reagent and yellow precipitate with Meyer reagent (Pardede et al., 2013).

Kuinon Test

A total of 5 mL of the experimental solution in the akaloid test stage (b) was taken, put into a test tube, then a few drops of NaOH 1N solution were added. When an intensive red color is formed, it indicates a quinone group compound.

Steroid/ triterpenoid Test

A total of 1 gram of simplicia powder or 0.2 grams of thick extract of meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera)* was macerated with 20 mL of ether for 2 hours (in a container with a tight lid and a dark room), filtered and taken 5 mL from the filtrate and then vaporized in an evaporation dish until a residue (residue) was obtained. Furthermore, 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid (Liebermann-Burchard Reagent) are added to the residue, forming a green color indicating the presence of steroid compounds or forming a color red violet indicates the presence of triterpenoid compounds.

Antioxidant Activity Test using DPPH (*2,2-diphenyl-1-picrylhydrazyl*) method

Thick extracts (meniran and kelor) were tested for antioxidant activity using the DPPH free radical reduction method and vitamin C and quercetin as positive controls.

Preparation of DPPH solution (0.4 mM)

Approximately 4 mg of DPPH (BM 394.32) was carefully weighed, then dissolved with methanol pro analysis up to 25 mL. The solution is placed in a light-insulated dark bottle.

Making a Blank Solution

A total of 1.0 mL of DPPH solution (0.4 mM) was pipetted into the flask, then methanol was added to the analysis to 5.0 mL, then homogenized.

Preparation of Test Solution

Approximately 10 mg of condensed extracts (meniran and kelor) were carefully weighed, then dissolved, in 10.0 mL of methanol pro analysis (1,000µg/mL), this solution is the parent solution. The parent solution was pipetted as much as 25µL, 50µL, 75µL, 100µL, 125µL into a 5.0 mL measuring flask to obtain a concentration of 5, 10, 15, 20, 25 µg/mL.

Preparation of vitamin C/quercetin Solution as a Positive Control.

Approximately 10.0 mg of vitamin C was carefully weighed, then put into a 10 mL measuring flask, added methanol pro analysis to 10.0 mL, homogenized so that a solution with a concentration of 1,000µg/mL (parent solution) was obtained. The master solution was pipetted as much as 2.5µL; 5.0µL; 7.5µL; 10µL; 12.5µL into a 5.0 mL measuring flask to obtain a concentration of 0.5; 1; 1,5; 2; 2.5 µg/mL.

Antioxidant Activity Test

Each test solution tube and comparator solution are added 1 mL of 0.4 mM DPPH solution and methanol pro analysis up to 5.0 mL, the tube is covered with aluminum foil and homogenized. The blank solution, test solution and positive control solution are immediately incubated in the incubator at 370C for minutes. After that, the absorbance was read on the visible spectrophotometry at a wavelength of 515.0 nm.

III. RESULTS AND DISCUSSION

Evaluation of Jelly Flawless Preparations

Uji organoleptis

Organoleptis is one of the most important factors that directly describe product quality. The resulting Flawless jelly should be homogeneous in color, undeformed in shape and surface, and pleasant in smell and taste. Organoleptical tests are also carried out to assess the characteristics of the preparations produced. The results of the organoleptis evaluation obtained can be seen in Fig 1 and Table 2.

A B C D E

Fig 1. (A) = Formula 1, (B) = Formula 2, (C) = Formula 3, (D) = Formula 4, (E) = Formula 5

Organoleptc Observatios	Formula				
	F1	F2	F3	F4	F5
Aroma	Strawberry	Strawbery	Strawbery	Strawbery	Strawbery
Color	Red	Red	Red	Red	Red
Taste	Sweet and sour	Sweet and sour	Sweet and sour	Sweet and sour	Sweet and sour
Texture	Very chewy	Supple	Quite chewy	Slightly chewy	Not chewy
Shape	Star Jelly	Star Jelly	Star Jelly	Star Jelly	Star Jelly

In the organoleptic test, the shape and texture are different in each formula. An increase in the amount of gelatin used causes the elasticity of the gummy candy In accordance with research that states that the lower the concentration of gelatin, the softer the *Flawless Jelly* produced. Meanwhile, the higher the concentration of gelatin, the softer the *Flawless Jelly* produced. The best formulation is Formula F1 with the author's preference for color, aroma, taste, shape, texture, and significance value 0.001–0.000, therefore ($p < 0.005$) at a significance level of 95% level, meaning that there is no meaningful or significant difference in formula 1.

Weight Uniformity Test

The preparation of gummies candies of meniran herbal extract is obtained by weight ± 1 gram for each one gummy. The results of the weight uniformity test of gummy candies were obtained that the average results for F1 to F5 met the weight uniformity requirements with none deviating from the range of column A (5%) and column B (10%). The percentage of the coefficient of variation given from each formula also still meets the requirements of the percent of the coefficient of variation in the uniformity of weight given according to the Indonesian Pharmacopoeia V (2020), namely CV ≤ 5%.

Uji pH

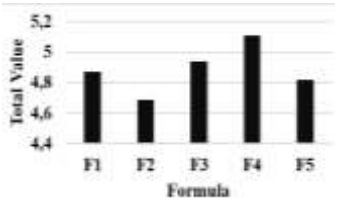

Fig 2. Test Results of Jelly Flawless Herbal Extracts of Meniran and Kelor

The overall data of the pH test results of jelly flawless meniran herbal extract was then analyzed using SPSS software. The normality and homogeneity test of the data showed that the data was distributed normally and homogeneously (p-value> 0.05). Furthermore, parametric analysis using One-Way ANOVA obtained a p-value of 0.520 > 0.05 at a significance level of 95%, which means that there is no significant difference in the pH of the flawless jelly preparation of meniran herbal extract between F1 to F5. This can be due to the fact that the pH value of flawless jelly is largely determined by the combination of extract and citric acid used. These two ingredients are used in equal concentrations for each formula, so the pH of the flawless jelly preparation obtained is significant between F1 and F5.

Moisture Content Test

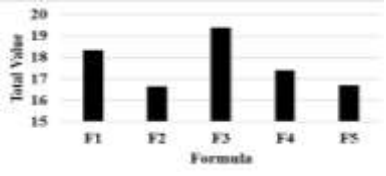

Fig 3. Results of Moisture Content of Jelly Flawless Meniran and Kelor Herbal Extracts

All data obtained from the results of the moisture content test were then analyzed parametrically using ANOVA, the p-value result was obtained <0.05 at a significance level of 95%, then continued the analysis with Post Hoc Tests LSD (Least Significant Different), the result was obtained that there was a significant difference in the moisture content of jelly flawles of meniran and kelor herbal extracts between the formulas F1 to F5, each formula F1 to F5 was significantly different from the moisture content of commercial products. This can be caused because the combination of gelatin and pectin and gelatin and single pectin used in F1 to F5 has the same total concentration of 20%, so the moisture content produced is significant, because the total amount of water contained in each formula is different. Based on the results obtained, it can be concluded that this study still meets the maximum moisture content requirements of jelly products according to Pharmacopoeia Ed VI (≤ 35%) and is not the same as commercial flawless jelly products that have been on the market.

Uji Kekerasan

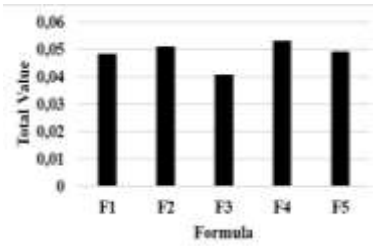

Fig 3. Results of Jelly Flawless Hardness Test of Meniran and Kelor Herbal Extracts

The overall data of the hardness test results was then analyzed using SPSS software. The results of ANOVA analysis showed that the p- value was 0.04 < 0.05 at a significance level of 95%, which means that there was a significant difference in the hardness of jelly flawless herbal extracts meniran and kelor between F1 to F5 and commercial products. Furthermore, the analysis was continued with Post Hoc Tests LSD (*Least Significant Different*) to see which formula had a meaningful difference from other formulas. The test results showed that it was significant with F1 to F5.

Elasticity Test

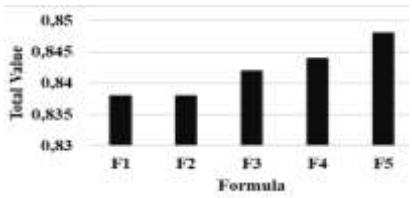

Fig 4. Results of Jelly Flawless Chewiness Test of Meniran and Kelor Herbal Extracts

The data from the overall results of the elasticity test was then analyzed using SPSS software. The normality and homogeneity test of the data used showed that the data was normally distributed with a p-value > 0.05. In the Kruskal waliss test annova test, the results showed that 0.009 which means a p-value of <0.05 there was a significant difference in the results of the elasticity test, The test results showed that it was significant with F1 to F5.

Phytochemical Tests

This phytochemical test aims to identify secondary metabolite compounds contained in meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera)* based on the type of extracting solvent. The phytochemical test data of the extract is presented in table 3 as follows:

Table 2. Phytochemical Test Results

Test Parameters	**Reactor**	**Observation**	**Result**
Tannins	1 – 2 drops of 1% iron(III) chloride reagent.	Green or blackish blue	+
Alkaloids	Wagner reagent, HCl2N and distilled water	Brownish yellow	+
Saponins	Hot water, cooled, shaken for 10 seconds, 1 drop of HCl 2N observed	Foam stabilizes 10 minutes	+
Flavonoids	Mg powder and four drops HCl 2%.	Orange-red	+
Quinon	Sodium Hydroxide 1 N	Red	+
Steroids	2 – 3 drops of anhydrous acetic acid, 1 – 2 drops of concentrated sulfuric acid	Green - Blue	+
Triterpenoids	2 – 3 drops of anhydrous acetic acid, 1 – 2 drops of concentrated sulfuric acid	Red-red-purple	-

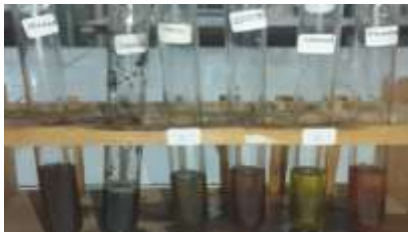

Fig 6. Phytochemical Test Results

Information:
Detected: (+),
Not detected : (-)

DPPH Antioxidant Test

Antioxidant activity testing was carried out to determine the antioxidant activity of 70% ethanol extract of meniran herb by determining the IC50 value. The IC50 value is the concentration of antioxidants that are able to inhibit 50% of free radicals. The results are shown in tables 4 and 5 with Linear Regression Equation = Y: -67.411x + 100.182

Table 3. Antioxidant Results of Meniran and Kelor

Concentration (µg/ml)	Log Concentration	Average	Abs Sample	% Inhibition	IC_{50} (µg/m)
5	0.699	0.747	0.638	6.861	**5.55**
10	1.000	0.616	0.507	25.985	
15	1.177	0.462	0.353	48.467	
20	1.301	0.350	0.241	64.818	
25	1.398	0.278	0.169	75.328	

Table 4. Antioxidant Result of Vitamin C

Concentration (µg/ml)	Log Concentration	Average	Abs Sample	% inhibisition	IC_{50} (µg/mL)
5	0.699	0.788	0.679	2.443	**2.78**
10	1.000	0.727	0.618	11.207	
15	1.177	0.565	0.456	34.483	
20	1.301	0.544	0.435	37.500	
25	1.398	0.417	0.308	55.747	

From the data above, it shows that the ethanol extract of 70% meniran and kelor herbs by using a wave length of 515 nm meets the quality standards of simplicia and extracts, the antioxidant activity test shows the results that the 70% ethanol extract has a strong antioxidant potential which is <50 (µg/mL).

Simplex Lattice Design (SLD)

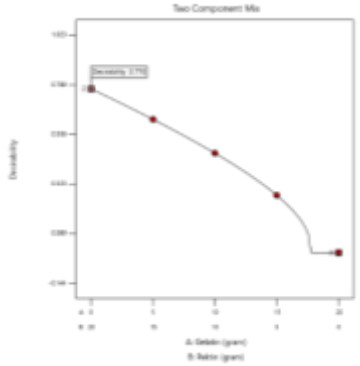

Fig 5. The optimal formula is selected from the contour plot

The Design Expert version 13 program will select the formula that is considered to have the highest desirability so that the optimal formula selected will produce the desired physical properties of the gel. A good desirability value is one close to 1, which is 0.770. The counterplot is shown in Figure 6. Based on the Countour plot in the figure, the optimum point is obtained which is marked with a box with the word Prediction along with the given desirability value, which is 1. The optimum point of the formula shows that the physical properties of the preparation are close to ideal, namely formula I with the addition of gelatin.

CONCLUSIONS

The optimum formula point shows that the physical properties of the preparation that are close to the ideal test based on the testing parameters of pH, water content, weight uniformity, elasticity and hardness are formula I with the addition of (0 pectin and 20 gelatin) desirability value of 0.770. The results of the antioxidant test on meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera)* extracts of 5.55 µg/mL are greater than the positive control, namely vitamin C of 2.78 µg/mL, indicating that the ethanolic extracts of meniran (*Phyllanthus niruri*) and kelor (*Moringa oleifera)* contain strong antioxidants based on this flawless Jelly preparation formulation can be consumed as an addition to antioxidants in the body

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