EVALUATION AND PHYSICAL STABILITY EFFERVESCENT POWDER OF OKRA LEAVES EXTRACT (*Abelmoschus esculentus*) AS AN ANTIDIABETIC SUPPLEMENT

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Abstrak

Diabetes Mellitus (DM) is a disease characterized by hyperglycemia and the most frequently encountered is type 2 which requires oral anti-diabetic therapy. Economic and socio-cultural factors also influence people in making their choice on alternative medicine. Okra (*Abelmoschus esculentus* (L.) Moench) is a medicinal plant containing the active substances flavonoids, α-cellulose and hemicellulose in its leaves which have anti-diabetic properties, especially type 2 DM. The aim of this research is to make a formula and determine the physical stability of the anti-diabetic supplement preparations in the form of effervescent powder from okra leaves extract. The method used is to make okra leaves extract effervescent powder in 3 formulations with a combination percentage of acid (tartaric and citric acid) and base (sodium bicarbonate) F1 = 27.87% : 26.8%, F2 = 26.05% : 28, 62%, F3 = 24.22% : 30.45%. The preparation evaluation includes organoleptic tests, flow rate, water content, effervescence time, pH and hedonics for 1 and 14 days of storage at cool temperatures (8-15°C), room temperature (15-30°C), and warm temperature (30-40°C). The results showed that variations in acid-base concentration had an effect on organoleptic physical properties (taste), flow rate, water content, effervescence time and pH. F3 shows the best formula and is the formula that respondents like most based on texture, color, flavour and taste parameters. Cool temperature (8-15°C) shows the best stability because its physical stability.

Kata Kunci*: Abelmoschus esculentus, Effervescent Powder, Diabetes Mellitus*

Background

Indonesia is a country with a tropical climate that is rich in wild flora and fauna. One of the rich flora that has many benefits is okra leaves. Okra leaves refer to the leaves of the okra plant (*Abelmoschus esculentus* (L.) Moench), which is also known by other names such as lady's fingers or gumbo. This plant belongs to the Malvaceae family. Okra is a popular vegetable in many tropical and subtropical locations, especially in Asia, Africa, and Latin America. Okra leaves are part of the plant that are also edible and have a number of nutritional benefits. Usually, okra leaves are served in soup or cooked form (Gemede, 2014; Watson dan Preedy, 2016). Okra leaves have health benefits. The 96% ethanol extract of Okra leaves contains flavonoids which act as antioxidants that inhibit oxidative stress due to free radicals which can damage pancreatic cells. An extract dose of 22.4 mg/20gBW in mice can reduce blood sugar levels by 67.68% (Aditama, 2019).

Diabetes mellitus (DM) is a disease characterized by hyperglycemia (Wells et al., 2016). Treatment of type 2 DM with Oral Anti-Diabetes (ADO) therapy which comes from chemicals, which in some people causes side effects, and anxiety. Apart from that, fear of surgery, dissatisfaction with conventional treatment, economic factors and socio-cultural factors also influence people in making their choice on alternative treatment. The appropriate formulation is aimed at increasing public interest in consuming okra leaves, one of which is formulating Okra leaves in the form of a nutritious drink such as effervescent powder as an antidiabetic supplement (Mohsen and Abdel, 2015; Watson and Preedy, 2016; Ardiyanto, 2017). Effervescent powder preparations are solid powder preparations consisting of a mixture of acids and bases which, when dissolved in water, will produce carbon dioxide gas, which has a sparkle taste like soda (Lieberman et al, 2013). Effervescent powder has the advantage of being able to dissolve quickly. In addition, its attractive color, flavour and taste make it easy to consume compared to other types of preparations (Egeten *et al.*, 2016).

Apart from the active substance, there are two additional substances which are characteristic of this preparation, namely acid and base. This research used two sources of acid and one source of base, namely citric acid, tartaric acid and sodium bicarbonate. The acid source gives the effervescent powder a sour taste to cover the bitter taste of the active substance, namely okra leaf extract (Sheskey, 2006).

Effervescent powder is made using a combination of tartaric acid and citric acid. The reason for this combination of acid components is because if you only use a single citric acid it produces a sticky mixture that is difficult to granulate, whereas if you only use a single tartaric acid it produces granules that easily coagulate (Forestryana et al., 2020). The effervescent powder formulation must meet the requirements, namely the combination of citric acid and tartrate must not exceed 30% (Cahyadi, 2008).

Effervescent powder formulations require a base component such as sodium bicarbonate. The aim of choosing sodium bicabronate is to increase solubility, improve taste, and not be hygroscopic (Forestryana et al., 2020). The sodium bicarbonate requirements required for effervescent powder range from 23.4% to 36.8% (Cahyadi, 2008). Therefore, by using appropriate acid and base sources, the physical properties of the effervescent powder will be maintained.

Determining the formula that has the best physical properties is carried out to test the physical stability of the preparation. The physical test of the preparation is carried out by observing and measuring several test parameters, namely organoleptic, flow rate, pH, water content, effervescent time and hedonic. The physical stability test is carried out by referring to the physical test of the initial preparation compared with preparations that have been stored at warm temperature (30-40^{o}C), room temperature (15-30^{o}C), cool temperature (8-15°C) for 14 days.

Physical stability is very important to ensure that the physical properties of the preparation remain the same after being produced and stored at different temperature variations. Several indicators of the physical stability of effervescent powder preparations include organoleptic stability, flow rate, pH, water content, effervescent time and hedonics, where no changes occur during storage and when used, and the characteristics and properties are the same as when they were originally made (Vadas, 2000).

Material and Methods

The research was carried out in November 2022 - September 2023 at the Pharmaceutical Technology Laboratory, Health Polytechnic of Jember. The research design is pre-experimental with a one shot case study design. The preparation of effervescent powder from okra leaves extract is made in 3 (three) formulas with varying acid concentrations (tartaric acid: citric acid): sodium bicarbonate, namely F1 = 27.87%: 26.8%; F2 = 26.05%: 28.62 %; F3 = 24.22%: 30.45%. Physical property tests are carried out using test parameters, namely organoleptic, flow rate test, water content, effervescent time, pH and hedonic. The best formula is tested for stability, namely stored at cool temperatures (8-15°C), room temperature (15-30°C), warm temperatures (30-40°C) by testing its physical properties on the 14th day after storage. The equipment used in the research were analytical scales (Fujitsu), mortar and stamper, 250ml glass beaker (Pyrex), test tubes (Iwaki), 10 ml measuring cups (Iwaki), 14 mesh, 16 mesh, 40 mesh sieves, stopwatch (Diamond), porcelain spoon, oven (Memmer), flowability tester, rotary evaporator (IKA HB 10 Basic), and digital pH meter (ATC), filter paper, aluminum foil.
The ingredients used are okra leaves, 96% ethanol (Inalab Utama), sodium bicarbonate (Bohr Chemical), tartaric acid (Merck), citric acid (Bohr Chemical), stevia sugar (Seduh Tea), PVP (Green PharmChem), melon flavoring.

Result and Discussion

Okra Leaves Preparation

The criteria for choosing okra leaves are that they must come from an okra plant that actively produces fruit, the leaves are fresh, and dark green in color. Okra leaves that meet these criteria are washed, weighed, and dried at 40° C until the dry leaves texture remains green. This temperature aims to maintain the flavonoid compounds contained in okra leaves. Active flavonoid compounds will be damaged at temperatures above 50°C (Ibrahim et al., 2015; Handayani and Sriherfyna, 2016). Then grind it with a blender and sift it to produce dry okra leaves powder weighing 482 grams. Extraction was then carried out via the maceration method using 96% ethanol solvent. The filtrate was then filtered and macerated again for 24 hours with five repetitions. After that, the results of the maceration were collected and evaporated using a rotary evaporator at a temperature of 40°C, resulting in a thick extract of okra leaves weighing 311.269 grams (Aditama, 2019).

Phytochemical Test

The yield value was calculated from the extraction results and identification of flavonoid compounds was carried out using 0.1 gram magnesium powder and 2 drops of concentrated HCl. The results are said to be positive if they show an orange, pink to red color in the solution (Munte dkk., 2021).

Preparation of Okra Leaves Extract Effervescent Powder

Manufacturing begins with weighing the ingredients according to the formulation. Grind the acid components (tartaric acid and tartaric acid) then sift with a 14 mesh sieve and dry in the oven at 40-50°C for 24 hours then sift again with a 16 mesh sieve (Mixture 1). The dry extract of okra leaves is flavored with powdered melon, then filtered through a 16 mesh sieve and baked in the oven at 40-50°C for 30 minutes (Mixture 2). Grind the base components (Sodium bicarbonate, stevia sugar, and PVP) until homogeneous then sift with a 16 mesh sieve and oven at 40-50°C for 24 hours (Mixture 3). In the final stage, mixtures 1, 2 and 3 are mixed until homogeneous, then sieved with a 40 mesh sieve to obtain effervescent powder, then stored in a tightly closed, airtight container.

Table 1. Okra Leaves Extract Effervescent Powder Formulation

Material	F1		F2		F3	
	%	gram	%	gram	%	gram
Okra Leaves Extract	43.4	43.4	43.4	43.4	43.4	43.4
Sodium bicarbonate	26.8	26.8	28.62	28.62	30.45	30.45
Tartaric acid	18.58	18.58	17.37	17.37	16.15	16.15
Citric acid	9.29	9.29	8.68	8.68	8.07	8.07
Stevia sugar	0.93	0.93	0.93	0.93	0.93	0.93
PVP	1	1	1	1	1	1
Melon flavoring	qs	qs	qs	qs	qs	qs
Total	100	10	100	10	100	10

Evaluation of The Physical Stability of Effervescent Powder

Organoleptic test

Organoleptic testing was carried out on the powder texture. Apart from that, color, odor and taste parameters were also carried out on effervescent preparations that had been dissolved in water. Testing was carried out by 3 respondents visually.

Flow Rate Test

The flow rate test was carried out by weighing 100 grams of effervescent powder then flowing it into the hoper and recording the time required for flow. This test was carried out 3 times.

Water Content Test

Water content testing was carried out by weighing 2 grams of effervescent powder then placing it in the oven for 5 hours at a heating temperature of 40°C then cooling it in a desiccator and weighing it (Egeten dkk., 2016).

Effervescent Time Test

The effervescent time test was carried out by weighing 10 grams and then placing it in a beaker filled with 200 ml of water. Calculate the dissolution time using a stopwatch (Egeten dkk., 2016).

pH test

pH testing is carried out by calibrating the pH meter (ATC) into a buffer solution of pH 4 (acid), pH 9 (alkaline), pH 7 (neutral), then weighing 4 grams of effervescent powder and then dissolving it in 150 ml of water. Measure the pH using a digital pH meter (Rizal et al., 2014).

Hedonic test

The subjects used in this test were 20 untrained respondents. The inclusion criteria for respondents were being willing to participate, being physically and mentally healthy (no color blindness, no flavour disorders, no psychological disorders).

Respondents were asked to rate their level of preference for texture, color, aroma and taste. The level of liking in the hedonic test is called the hedonic scale. This research uses a hedonic scale of 1 to 5 and is also interpreted in more detail according to Suryono et al., (2018), namely 0 - 1.0 (very dislike); 1.1 - 2.0 (dislike); 2.1 - 3.0 (somewhat like); 3.1 - 4.0 (likes); 4.1 - 5.0 (like very much).

Data analysis

Data from organoleptic test research on effervescent powders were compared with literature parameters. Data from tests on the physical properties of flow rate, water content, effervescent time, pH and hedonics were analyzed statistically using Statistical Product Services Solution (SPSS) Version 26. The data were tested for normality using the Shapiro Wilk method. Testing continued using One Way Anova (Analysis of Variant). The results of the hedonic test data were analyzed using the Univariate method and continued using post hoc Duncan.

Results and Discussion

Results of Okra Leaf Extraction

The process of extracting okra leaves resulted in an extract of 311.269 grams. The yield calculation results are shown in **table 2**.

Table 2. Yield of Okra Leaf Extract (*Abelmoschus esculentus* (L.) Moench)

Simplicia Powder Weight	Weight of Extract Results	Yield Percentage
482 gram	311.269 gram	64.57%

The results of phytochemical screening showed that the ethanol extract of okra leaves positively contained flavonoid compounds. Positive results for flavonoids in the extract showed a change in color to orange and red after being given concentrated HCl and 0.1 g of magnesium powder.

Organoleptic Test Results of Okra Leaves Extract Effervescent Powder

Table 3. Organoleptic Results of Okra Leaves Extract Effervescent Powder

Organoleptic	Formula 1	Formula 2	Formula 3
Texture	Fine	Fine	Fine
Flavour	Medium Melon	Medium Melon	Medium Melon
Taste	Highly Acid and Low Fizzy	Sour and Medium Fizzy	Slightly Sour and Highly Fizzy
Color	Green	Green	Green

The organoleptic test results are shown in **table 3**. All formulas produce preparations that have a smooth texture, medium melon odor, green color with different variations of acid and fizzy, due to the different concentrations of acid and base used.

Flow Rate Test Results

Table 4. Effervescent Powder Flow Rate Results

Formula	Replication			Average ± SD
	1	2	3	(g/s)
I	24.39	24.03	24.44	24.29±0.22
II	23.75	23.52	23.58	23.62±0.12
III	18.48	18.41	19.19	18.69±0.43

The results of the effervescent powder flow rate test can be seen in **table 4**. The three formulations are included in the very good flow rate category, because they have a flow rate value of > 10 g/s. F1 has a very good flow rate value because it has the largest flow rate value. Flow rate is influenced by particle shape, size and density (Hayaza et al., 2019). Statistical analysis of the normality of the flow rate test showed a significance of 0.004 ($p<0.05$), which means the data was not normally distributed. Data processing was continued using the Kruskal Wallis Test and obtained a significance value of 0.027 ($p<0.05$), which means that there is an influence of variations in acid base concentration on the flow rate value between the three formulas. Next, to find out which formula has an effect, the Post Hoc test continues. The results of F3 are significantly different from F1, this can be proven by a significance value of 0.007 ($p<0.05$). The significant difference in effect is because the concentration of tartaric acid in formula 1 and formula 2 is greater than in formula 3, tartaric acid has a greater density with a mass of 1.76 g/cm3 than citric acid (1.542 g/cm^3) so that the powder contains More tartaric acid will have a greater density (Hayaza et al., 2019). If the density is greater, the molecular weight will be greater so it will flow more easily due to the greater gravitational force (Anshory, 2007).

Water Content Test Results

Table 5. Water content results

Formula	Replication			Average ± SD (%)
	1	2	3	
I	4.30	4.25	4.27	4.27±0.03
II	3.62	3.66	3.60	3.63±0.03
III	2.87	2.80	2.81	2.83±0.04

These results show that the water content of all formulas meets the requirements for effervescent powder water content, namely <5% (Egeten et al., 2016). High water content is related to the effervescent reaction, if the water content is too high it will trigger a premature effervescent reaction so a low water content is needed (Elfiyani dkk., 2014).

Statistical analysis of the normality of the water content test showed a significance of 0.070 ($p>0.05$), which means the data was normally distributed. Next, the average water content test data from the three formulas were analyzed using One Way Anova and a significance value of 0.000 ($p<0.05$) was obtained, which means that there was an influence of variations in acid base concentration on the water content value between formulas. Next, to find out which formula has an effect, the Post Hoc Tukey test is continued. The results are that F1 is significantly different from F2 and F3, and F2 is significantly different from F3. This can be proven by all significance values of 0.000 ($p<0.05$).

This significant difference in effect is because the citric acid content in F3 is lower compared to F1 and F2. Citric acid is hygroscopic so it has the potential to absorb water vapor in the air. According to research by Forestryana et al., (2020), the lower the citric acid content in the formula, the lower the water content. Determining the formula that has the lowest water content value can be seen from the smallest subset value, namely F3, because it has the smallest subset value, namely 2.82.

Effervescent Time Test Results

Table 6. Effervescent Time Results

Formula	Replication			Average ± SD (minute)
	1	2	3	
I	3.15	3.16	3.17	3.16±0.01
II	2.80	2.80	2.80	2.80±0.00
III	1.17	1.16	1.15	1.16±0.01

The three formulas meet the effervescent time requirements for effervescent powder, namely <5 minutes (Kailaku et al., 2012). F3 has a faster effervescent time compared to F2 and F1, because F3 has more sodium bicarbonate components, thus producing more CO_2 gas. This is because the more CO_2 gas produced, the faster the effervescent time required (Forestryana dkk., 2020).

Statistical analysis of the normality of the late time test obtained a significance value of 0.003 ($p<0.05$), meaning the data was not normally distributed. Data processing was continued using the Kruskal Wallis Test, obtaining a significance value of 0.024 ($p<0.05$). This shows that there is an influence of acid base variations on the effervescent time in the three formulas. Next, to find out which formula has an effect, the Post Hoc test is continued. The results show a sig value of 0.006 ($p<0.05$), which means there is a significant difference between F3 and F1. This means that the effervescent time of F3 is faster than F1. Determining the formula that has the fastest flow time value can be seen in the smallest ranking value in the pairwise comparisons of formula image, namely F3.

pH Test Results

Table 7. pH test results

Formula	Replication			Average ± SD
	1	2	3	
I	4.5	4.6	4.7	4.6±0.1
II	5.2	5.3	5.4	5.3±0.1
III	6.1	6.2	6.3	6.2±0.1

Based on observations, F1 and F2 have a lower pH compared to F3. So it can be said that the pH of F1 and F2 does not meet the pH requirements for effervescent powder, namely that it must be in the range of 6 to 7 (Kumullah, 2016). The results of pH measurements show that the ratio of acid concentrations has an effect on the pH value of okra leaves extract effervescent powder. This effect is because sodium bicarbonate will react with acid and water to release free Na^+ ions. These ions bind with organic acids and form sodium bicarbonate salts so that the activity of H^+ ions in organic acids is reduced (Wahyuningsih dkk., 2022).

Statistical analysis of the normality of the pH test showed a significance of 0.234 ($p>0.05$), which means the data was normally distributed. Data processing was continued using the One Way Anova method, obtaining a significance value of 0.000 ($p<0.05$), which means that there is an influence of variations in acid base concentration on the physical properties of pH. Next, to find out which formula has an effect, the Post Hoc Tukey test is continued. The results are that F1 is significantly different from F2 and F3, and F2 is significantly different from F3. This can be proven by all significance values of 0.000 ($p<0.05$). F3 is a formula that meets the pH test requirements, because it meets the range of pH test requirements.

Hedonic Test Results

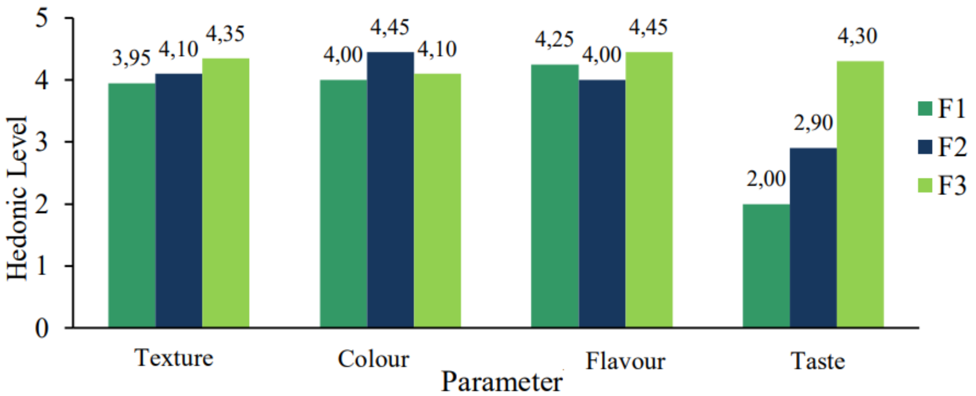

Figure 1. Hedonic Level Results

This hedonic test aims to determine the level of respondents' liking for the product produced. The test parameters used include the texture of the effervescent powder, color, aroma and taste of the effervescent preparation after dissolving it in water. The test method uses scoring with a total of 20 respondents. Statistical analysis of the hedonic test using the Univariate method for assessing texture, color, aroma and taste parameters showed that there were no significant differences between the test formulas with significance values (p=0.220), (p=0.114), (p=0.121) and (p =0.000).

Testing continued using the Post Hoc Duncan test. As a result, F1, F2 and F3 occupy different subset columns. F1 has a subset value of 2.00, F2 has a subset value of 2.90, and F3 has a subset value of 4.30. Determining the formula that is most popular is by looking at the largest subset value, namely F3. The largest subset value is 4.30 which is included in the very like rating.

Stability Test Results

The stability results use formula 3 as the best formula in terms of physical properties. Stability tests were carried out for 14 days at cool temperatures (8 - 15 °C), room temperatures (15 - 30 °C), warm temperatures (30 - 40 °C).

Table 8. Stability Test Results

Parameter	Day 0			Day 14		
	Cool temperatures	**Room temperatures**	**Warm temperatures**	**Cool temperatures**	**Room temperatures**	**Warm temperatures**
Organoleptic						
a. Textur	Fine	Fine	Fine	Fine	Clot	Fine
b. Flavour	Medium Melon	Medium Melon	Medium Melon	Medium Melon	Medium Melon	Medium Melon
c. Taste	Slightly sour and High Fizzy	Slightly sour and High Fizzy	Slightly sour and High Fizzy	Slightly sour and High Fizzy	Slightly sour and High Fizzy	Slightly sour and High Fizzy
d. Color	Green	Green	Green	Green	Green	Green
Flow Rate (g/s)	18.69±0.43	18.69±0.43	18.69±0.43	18.79±0.05	18.23±0.1	18.55±0.04
Water content (%)	2.83±0.04	2.83±0.04	2.83±0.04	2.81±0.01	3.56±0.06	2.23±0.02
Effervescent time (minute)	1.16±0.01	1.16±0.01	1.16±0.01	1.23±0.04	4.25±0.17	3.17±0.02
pH	6.20±0.1	6.20±0.1	6.20±0.1	6.33±0.15	6.27±0.06	6.10±0.1

Organoleptic Stability Test Results

The organoleptic odor of the effervescent powder before storage showed a medium melon odor and remained stable for 14 days of storage at cool, room and warm temperatures. The organoleptic test results for the taste of the effervescent powder before storage showed that it was slightly acidic and highly fizzy and remained stable for 14 days of storage at cool, room and warm temperatures. The organoleptic color of the effervescent powder showed stable results, before and after storage for 14 days at cool, room and warm temperatures.

Organoleptic observations effervescent powder before storage has a fine texture because it is sieved using a 16 mesh sieve. Storage for 14 days at cool and warm temperatures showed no change in texture, however at room temperature there was a change in clot texture. This can be caused by the relative humidity of the room being 60%, while the relative humidity for effervescent powder is 25%, so that the water content of the powder will change to an equilibrium condition between the water in the powder and the water in the indoor air (Fausett, 2000; Sheskey et al., 2006).

Flow Rate Stability Test Results

Test data for the stability of the flow rate of effervescent powder before and after storage at cool, room and warm temperatures shows that it is stable at all storage temperatures. This shows that there is no significant effect of temperature variations before and after storage with a p value of >0.05, namely cool temperature 0.752, room temperature 0.271 and warm 0.587.

Water Content Stability Test Results

Variations in storage at room and warm temperatures have a significant effect on the stability of the water content of Okra leaves effervescent powder. This is shown by a p value <0.05 at room temperature 0.005 and warm 0.003. Cool temperature shows the best storage temperature because it maintains the stability of the preparation significantly with a value of 0.204 (p>0.05).

Effervescent Time Stability Test Results

Effervescet time Okra leaves effervescent powder is unstable in storage at room temperature and warm. This is shown by a significant change in dissolution time with a p value <0.05, namely room temperature 0.000 and warm 0.001. Okra leaf effervescent powder preparation was significantly stable when stored at cool temperatures with a value of 0.252 (p > 0.05).

pH Stability Test Results

Okra leaves effervescent powder showed a significantly stable pH p > 0.05 when stored at cool temperatures of 0.456, room 0.529 and warm 0.529. This shows that variations in storage temperature have no effect on the physical stability of the pH of Okra leaves effervescent powder.

Conclusion

Varying acid and base concentrations in effervescent powder formulations affect the organoleptic physical properties (taste), flow rate, water content, effervescent time and pH but do not affect the organoleptic physical properties (texture, odor and color). Formula 3 is an effervescent powder with the best physical properties. Variations in storage temperature affect the physical stability of effervescent time and water content, but do not affect the physical stability of organoleptics, pH and flow rate of effervescent powder. Cool temperature is the best storage temperature for Okra leaf effervescent powder.

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