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Effervescent Powder Formulation of Ethanol Extract of African Leaves (*Vernonia amygdalina*) as an α-amylase Inhibitor

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Abstract

Vernonia amygdalina or African leaves are reported to have many biological activities, including antidiabetic. However, there are still many people who have not used this plant as an antidiabetic treatment. This research aims to create a medicinal preparation formula in effervescent form with the main ingredient being African leaves. Testing of the dosage formula includes organoleptic tests, water content, pH, and dispersing time. The research results showed that one of the three formulas had better stability after 7 days of storage. After 7 days of storage, the color of the formula became lighter, the pH value changed slightly (7-8), the water content was 2.3% and the dispersion time was 2.00 minutes. This formula can be used as input for further research regarding testing its activity as an antidiabetic through inhibiting alpha-amylase in vitro.

Keywords : herbal, diabetes, simplicia, extraction

INTRODUCTION

Vernonia amygdalina or African Leaf is a wild plant that is very easy to grow and reproduce around us, but many people still ignore its benefits. Several studies have proven that this plant has many biological activities. African leaves are reported to contain many active compounds. The active compounds identified in this leaf extract include flavonoids, phenols, tannins, alkaloids, and saponins (Yunitasari *et al.*, 2022). The diversity of active compounds in this plant causes the plant to have several biological activities. The biological activities possessed by this plant include anti-inflammatory (Quasie *et al.*, 2016), antibacterial and antioxidant (Habtamu and Melaku, 2018), and antidiabetic (through the mechanism of inhibiting α-amylase and α-glucosidase) (Anh *et al.*, 2021). Inhibition of α-amylase is one of the mechanisms for treating type two diabetes mellitus (T2DM) (Li *et al.*, 2017).

Indonesia is one of the countries with the most T2DM sufferers in the world (after China and India) (Ogurtsova *et al.*, 2022). T2DM treatment is aimed more at controlling blood sugar levels, one of which is controlling glucose intake. Controlling glucose intake can be done by inhibiting the action of the α-amylase enzyme. The α-amylase enzyme is an enzyme that catalyzes the hydrolysis of 1-4 glycosidic bonds in starch and polysaccharides such as amylose into maltose and other simple sugars (Narita and Inouye, 2015).

The currently used treatment for T2DM is synthetic drugs, such as metformin, sulfonylureas, meglitinides, and so on. However, synthetic medicines or modern medicines are considered less safe than traditional medicines because they have relatively more side effects (Sumayyah and Salsabila, 2017). The great potential of the Vernonia amygdalina plant (especially as an antidiabetic) should be explored further for its utilization. An effort to explore the use of the Vernonia amygdalina plant is by making a medicinal preparation formulation. One dosage form that can be used is effervescent (BPOM, 2019).

Effervescent is a solid preparation of traditional medicine, made from certain extracts and/or simplicia, containing sodium bicarbonate and organic acids which produce gas bubbles (carbon dioxide) when put into water (BPOM, 2019). Effervescent can be presented in tablet form (Sholikhah *et al.*, 2018), powder (Maryam *et al.*, 2022), and granules (Syaputri *et al.*, 2023). Of these three dosage forms, the powder form has a larger surface area, so it is expected to have a faster absorption capacity than the other forms.

African leaves are known as "bitter leaves". This is because African leaves will produce a bitter taste when consumed. The bitter taste of plant extracts or powders can be reduced by the presence of fillers, such as maltodextrin (Permana *et al.*, 2012). So, it is hoped that by adding sodium bicarbonate and organic acids, this medicinal preparation from African leaves will not produce a bitter aftertaste. Medicinal preparations in the form of effervescent powder can provide the advantage of producing carbon dioxide gas which gives a fresh taste like soda water (Syamsul and Supomo, 2014).

Based on the background above, the basic aim of this research is to produce a medicinal preparation formulation in the form of effervescent powder with the main ingredient being Vernonia amygdalina leaf extract. This research is the basis for supporting further research, namely the availability of herbal medicine for diabetes mellitus.

METHODS

This type of research is experimental. The variable in this research was making an effervescent powder formulation of African leaf extract (Vernonia amygdalina) by varying the amount of African leaf extract and sucrose. In this research, a ratio of acid and base was 1:1.

Equipment

The tools used in this research include a maceration vessel, rotary evaporator (Heidolph), analytical balance (centarus scale), sieve no. 45, blender (Philips), universal pH, thermometer, oven (UN110), stopwatch, aluminum tray, mortar pestle and also other glass tools (herma).

Material

The materials used in this research include African leaves, pro-analyst ethanol, sodium bicarbonate (disintegrating agent), citric acid and tartaric acid (adhesive agent), lactose (filler agent), sucrose (sweetener agent), CMCNa (binder agent), and essence (aroma and taste enhancer).

Method

1. Sample preparation

The African leaves used in the research were cultivated and harvested from Ponorogo Regency, East Java. After being harvested, African leaves go through wet sorting, washing, slicing, chopping, drying, and dry sorting. The drying process uses the airing method in the shade. The powder obtained was subjected to microscopic testing to ensure that the leaves used as research samples were truly African leaves or Vernonia amygdalina. This stage is a way to process sample determination.

2. Preparation of African leaf extract

African simplicia leaves are ground using a blender and sifted. The African leaf powder was then extracted using the maceration method and using pro-analyst ethanol solvent. The solvent was added to a height of 1 cm above the sample surface (Sholikhah et al., 2018). After the maceration process, the filtrate is filtered and continued with the evaporation process with a rotary evaporator at a temperature of 40^{o}C. This is done to remove the solvent. As a result of evaporation, a thick extract of African leaves was obtained and the yield was calculated (Hasan *et al.*, 2022).

$$\% \text{ yield} = \frac{\text{weight of the extract obtained}}{\text{the weight of the extracted simplicia}} \text{ x } 100\%$$

3. Preparation of African leaf extract effervescent powder formulation
   a. Ingredient formula

In this research, three formulas of African leaf ethanol extract effervescent powder were made (F1, F2, and F3). The three formulas are differentiated by the amount of extract and sucrose. The aim of varying these three formulas is to minimize the bitter taste and distinctive aroma of African leaves,

as well as to have good physical quality. The formulations of the three formulas can be seen in Table 1.

Table 1. Three African Leaf Ethanol Extract Effervescent Powder Formulas

No.	Ingredient	F1	F2	F3
1	Ethanol extract of African leaves	3	5	7
2	Sodium bicarbonate	10%	10%	10%
3	Citric acid	7,5%	7,5%	7,5%
4	Tartaric acid	4,5%	4,5%	4,5%
5	Lactose	10%	10%	10%
6	Sucrose	58,5%	56,5%	54,5%
7	CMCNa	5%	5%	5%
8	Esscence	q.s	q.s	q.s

b. Mixing ingredients

The thick extract of African leaves, acid ingredients (citric acid and tartaric acid), and additional ingredients (lactose, sucrose, and CMCNa) are weighed according to a predetermined formula, except for additional ingredients which are only half the formula. All the ingredients are mixed until homogeneous and placed in an oven at 65^{o}C for 5 minutes, then the results are placed on tray 1. Next, the thick extract, sodium bicarbonate, and half the formula of the additional ingredients are weighed again. All these ingredients are also mixed until homogeneous and the oven process continues at 65^{o}C for 5 minutes, and the results are placed in tray 2. The next stage is to mix the results in trays 1 and 2, stirring until homogeneous and smooth using a pestle and mortar. As a result of mixing, add enough essence and continue the oven process at 40^{o}C for 5 minutes. The resulting powder is stored in a plastic clip.

4. Physical test of African leaf ethanol extracts effervescent powder

The physical tests carried out after the effervescent preparation is formed (day 0) include organoleptic tests, pH tests, dispersion time, and water content tests. Apart from that, this research also carried out stability tests. The stability test aims to determine the shelf life of the preparation. The way to carry out a stability test is to carry out organoleptic, pH, dispersion time, and water content tests on the 7th day. The organoleptic test carried out is to observe the color, taste, and odor of the preparation. Testing the water content of the preparation is carried out by weighing the preparation and continuing the drying process in an oven at a temperature of 40^{o}C until it produces a constant weight. A good water content for effervescent powder is around 5-7% (Septianingrum et al., 2019). Uji kelarutan dilakukan dengan cara melarutkan serbuk effervescent ke dalam aquades dan dihitung waktunya sampai larut total. Larut total ditandai dengan berhentinya gelembung udara yang ditimbulkan oleh sediaan. Nilai pH yang baik untuk larutan effervescent adalah mendekati netral (Kumullah, I., 2016).

5. Data analysis

The data analysis carried out was a qualitative and quantitative description. Qualitative descriptions explain the results of microscopic testing, organoleptic tests, and stability tests, while quantitative descriptions explain the results of pH tests, dispersion time, and water content.

RESULTS AND DISCUSSION

The drying process can produce about one kilogram of dry African leaf powder from eleven kilograms of wet African leaf. For dry African leaf powder, water content testing was carried out. The results of the test showed that the water content of dried African leaf powder (11.38%) still exceeded the quality requirements for simplicia, namely <10%. Simplicia with water content that exceeds the quality requirements for simplicia will encourage microbial growth (Utami *et al.*, 2017), so the simplicia will rot easily. The high-water content in dried African leaf powder is due to the drying process being carried out during the rainy season, which causes environmental conditions to often be humid and room temperatures to be unpredictable. In terms of the color of African leaf powder, it can be seen in Figure 1. Powder with a water content of more than 10% has a blackish-green powder color.

Figure 1. African Leaf Powder

Microscopic tests showed that fragments were found in the samples to be studied, including the lower epidermis with stomata, parenchyma with rosette-shaped calcium oxalate crystals, leaf mesophyll and scale hairs, and covering hair (Figure 2). There are several characteristic fragments of African leaf simplicia, including the lower epidermis with stomata, leaf mesophyll and scale hairs, sclerenchyma, covering hairs, transport bundles with mesh-type thickenings, and parenchyma with rosette-shaped calcium oxalate crystals (Menkes RI, 2017). So, the sample studied was identified as a species of Vernonia amygdalina.

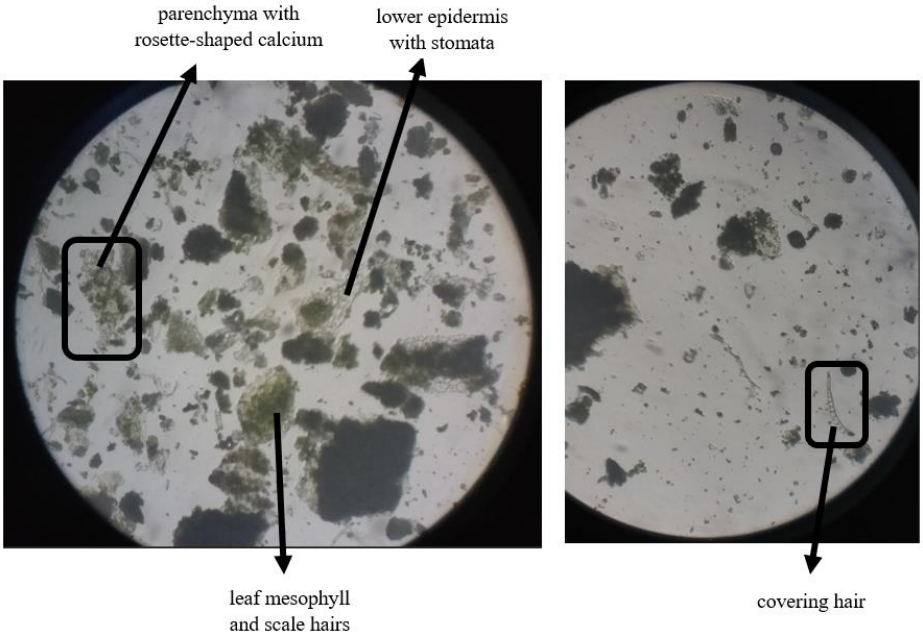

Figure 2. Fragments in African simplicia leaf powder

Dried African leaf powder was extracted using the maceration method and evaporated using a rotary evaporator. The maceration results showed that the yield of ethanol extract from African leaves (Figure 3) was 9.3%. This yield is from 500 grams of dry African leaf powder. The yield value shows the amount of bioactive content (Dewatisari *et al.*, 2018). The yield values obtained in this study show that the bioactive content extracted using the maceration method and ethanol solvent is still relatively small. In this research, at the beginning of the maceration process, 2 liters of ethanol solvent were poured in and after 4 days of the maceration process, 1 liter of filtrate was obtained. This difference is thought to be due to the evaporation of the solvent during maceration so that the extraction process cannot be carried out optimally. The yield value of 9.3% does not meet the yield requirements for the thick extract of African leaves. The requirement for yield of thick African leaf extract is not less than 11.8% (Menkes RI, 2017).

Figure 3. Ethanol extract of African leaves

The preparation of effervescent powder preparations from ethanol extract of African leaves is prepared in three formulas. The three formulas from the results of the effervescent powder formulation on day 0 can physically be seen in Figure 4. The three formulas are differentiated by the amount of extract and sucrose. Judging from the amount of extract, F1 < F2 < F3 and in terms of the amount of sucrose F1 > F2 > F3. The physical tests of the three effervescent powders were carried out by organoleptic testing, water content, pH, and dispersion time. This physical test will also be carried out again on the 7th day to obtain data regarding the stability of the preparation. The physical conditions of the three formulas on day 7 (Figure 5) were different in terms of organoleptic.

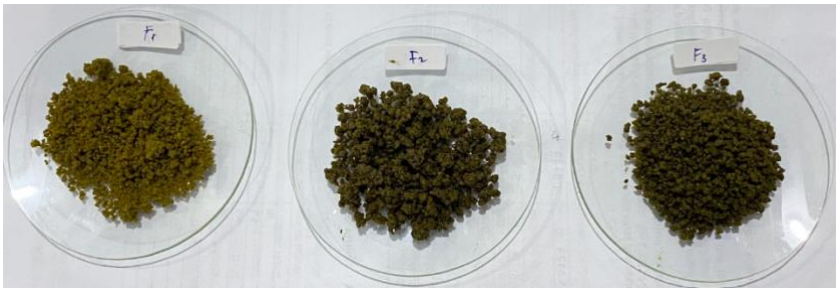

Figure 4. Three effervescent powder formulas for ethanol extract of African leaves on day 0

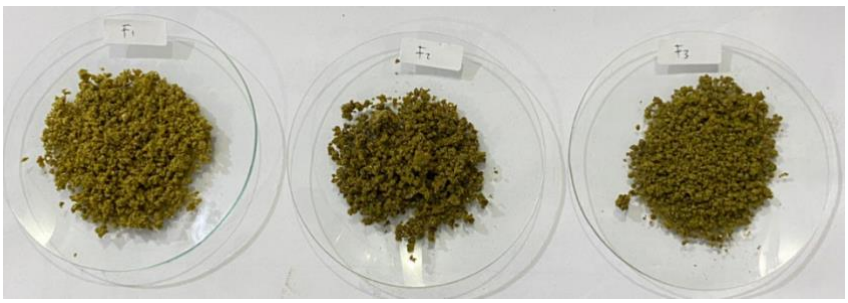

Figure 5. Three effervescent powder formulas for ethanol extract of African leaves on day 7

For organoleptic tests, the effervescent powder is assessed in terms of color, taste, and odor. The results of organoleptic testing on days 0 and 7 can be seen in Table 1. On day 0, the color of F1 was lighter than F2 and F3. Based on this organoleptic test in terms of color, it can be concluded that the more extract used will affect the color of the preparation (the more extract, the darker the color of the preparation). Of the three formulas, F1 and F2, it can be stated that there is no distinctive smell from African leaves. This is because African leaf extract has a distinctive characteristic (Menkes RI, 2017). Organoleptic tests in terms of taste, the addition of other components to this effervescent formula can cover the bitter taste of African leaves. However, F3 still has a slightly bitter taste. This is because F3 contains the most African leaf extract compared to the other two formulas. The stability of the three effervescent powder formulas changed on day 7. Formula 2 and Formula 3 experienced significant changes in color, odor, and taste. However, formula 1 experienced minimal changes in condition, namely slight changes in color and smell, while the taste did not change.

Table 1. Organoleptic test results on day 0 and day 7

No.	organoleptic test	Testing day-	F1	F2	F3
1	Color	0	yellowish green	blackish green	blackish green

		7	increasingly yellowish green	yellowish green	yellowish green
2	Smell	0	odorless	odorless	weak characteristic odor
		7	smells typical of African leaves	smells typical of African leaves	smells typical of African leaves
3	Flavor	0	Sour	Sour	slightly bitter sour
		7	Sour	slightly bitter sour	slightly bitter sour

The water content for the three effervescent powder preparations can be seen in Table 2. Testing the water content of effervescent powders can be concluded that all formulas meet the water content quality requirements for effervescent preparations. Quality requirements for water content in effervescent preparations ≤ 5% (BPOM, 2019). This test is carried out to determine the concentration of water in the preparation. Water content that is too humid can cause the effervescent powder to clump and mold quickly, making it unusable (Septianingrum *et al.*, 2019). After 7 days of storage, there was an increase in the water content of the effervescent powder preparation. This shows that the longer the storage of the preparation, the more water content in the preparation increases. This condition can cause spoilage or damage to the preparation. However, after 7 days of storage, it showed that the conditions of F2 and F3 still met the preparation quality requirements, i.e. less than 5%. Formula 1 exceeds the preparation quality requirements because it is more than 5%.

Table 2. Water content of the three formulations

Testing day-	F1	F2	F3
0	1,1%	1,8%	1,2%
7	9,1%	2,3%	4,6%

The pH testing of the effervescent powder preparations can be seen in Table 3. From the test results, it can be concluded that the pH of the three formulas on day 0 is close to neutral (pH 6-7). If the pH of the effervescent preparation is between 5-7, then the preparation is better (Kumullah, I., 2016). Effervescent preparations that are too acidic or alkaline will cause stomach irritation and produce a bitter taste (Septianingrum *et al.*, 2019). So, all African leaf extract effervescent powder formulas meet the pH effervescent quality requirements. On day 7, there was a change in the pH of the three formulations, i.e. to 7-8. Under these conditions, stability begins to decrease after day 7 (mainly a pH problem).

Table 3. pH value of effervescent powder on days 0 and 7

No.	pH test	0	7
1	F1	6-7	7-8
2	F2	6-7	7-8
3	F3	6-7	7-8

Dispersion time is one of the physical properties of effervescent preparations which is typically by dissolving the preparation in water and will cause an acid-base reaction which is characterized by the appearance of CO_2 gas and causing the dissolution of the effervescent preparation (Egeten *et al.*, 2016). The dispersion time of the three formulas can be seen in Table 2. On day 0, the three formulas meet the quality requirements for the dispersion time of an effervescent preparation. The quality requirement for the dispersion time of a preparation is no more than 5 minutes (Safitri, 2021). However, for the three formulas, in terms of dispersion time, F1 is better than the other formulas. On day 7, the dispersion time accelerated (Table 4). This means that the dispersion preparation becomes better after adding water (Oktavina and Imtihani, 2023).

Table 4. Dispersion time of the three formulas on day 0 and day 7

No.	dispersion time	0	7
1	F1	2,16 minutes	1,55 minutes
2	F2	2,22 minutes	2,00 minutes
3	F3	2,27 minutes	1,55 minutes

Based on organoleptic tests, pH tests, dispersion time, and water content, the stability of formula 2 is better than the other 2 formulas. This is indicated by the color getting brighter and a slight change in smell and taste. There was a slight change in the pH value (still around neutral conditions), the

water content was less than 5%, and the dispersion time became faster. This condition requires more attention to the storage location of effervescent powder.

CONCLUSION

Based on the tests that have been carried out, i.e. organoleptic tests, water content, pH, and dispersing time, the three formulas approach the quality requirements of an effervescent preparation. However, judging from the stability of the preparation condition after 7 days of storage, formula 2 has better stability than the other 2 formulas. The results of this research can be used as a basis for further research for in vitro testing of preparations.

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