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Original Article

ANTIOXIDANT ACTIVITY OF EFFERVESCENT GRANULES FROM KIRINYUH LEAVES (*CHROMOLAENA ODORATA* (L.) R. M. KING and H. ROB) AND LEAF OF MAREME (*GLOCHIDION ARBORESCENS* BLUME)

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ABSTRACT

Objective: The objective of the study was to prepare and evaluate the formulation of the granules effervescent from kirinyuh and mareme leaves as an antioxidant.

Methods: The method of making granules with the wet granulation method. The granulation method was used for making granules, meanwhile, the evaluations of the granules was included organoleptic tests, water content, solubility time, pH of the preparation, compressibility, flow time angle of repose and antioxidant activity.

Results: The results showed that combination of kirinyuh and mareme as effervescent showed fulfilled all the test criteria, however, demonstrated weak antioxidant activity.

Conclusion: The three formulations of effervescent granule preparations from kirinyuh leaves and mareme leaves have very weak antioxidant activity

Keywords: Antioxidant, Effervescent, Granule, Kirinyuh, Mareme

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INTRODUCTION

Indonesia is one of the country with a largest diversity of plants which have various types of benefits and are widely used by the community as food, agriculture, and medicinal ingredients or traditional medicine. Kirinyuh is a plant from the Asteraceae family, according to Saputra (2017), kirinyuh leaves showed powerful antioxidant properties [1]. In while the mareme plant is a Euphorbiaceae family, according to Indra (2019) found that mareme leaves had a potential source of natural antioxidants [2].

Antioxidants are substances, vitamins, minerals, or enzymes that protect body cells from oxidative damage by free radicals. These free radicals are radiation, pressure from the environment, or as a result of normal processes of mitochondria and cells [3]. Free radicals are reactive oxidants that have one or even more unpaired electrons on the outer orbital and attack cellular components such as lipoproteins, proteins, DNA, RNA, and carbohydrates [4].

Granules are preparations resulting from the granulation process such as a processing of small powders and mixing them together into a larger form in the form of lumps, and allowing the granules to flow freely [5]. Effervescent is a preparation that can dissolve or disperse quickly in water and produce air bubbles as a result of the release of carbon dioxide gas that mask an unpleasant taste [6].

The formulation of the effervescent granule preparation and its evaluation will be carried out with the ingredients to be used. It is expected the combination of the dried extracts of the two leaves can be formulated into effervescent granules showed good physical properties and demonstrated antioxidant activity.

MATERIALS AND METHODS

Materials

Kirinyuh leaf extract and mareme leaf extract were obtained from Tasikmalaya City, West Java, Indonesia. The other materials including ascorbic acid (Merck, Germany), citric acid (Merck, Germany), sodium bicarbonate (Brataco, Indonesia), lactose (Brataco, Indonesia), aerosil (Wacker, China), PVP K-30 (Brataco, Indonesia), alcohol (Brataco, Indonesia), melon essence (Robindo, Indonesia), aqua dest, ammonia, chloroform, HCl, Mayer, Dragendorff and Wagner reagent Mg powder, amyl alcohol, $FeCl_3$, NaOH, acetic acid, sulfuric acid, methanol, DPPH, all materials were purchased from Sigma Aldrich.

This study use flow tester, analytical balance (Mettler Toledo®), micropipette (Finnpipette F2®), stopwatch, UV-Vis spectrophotometry (Agilent Technologies), pH meter (OHAUS Starter 5000®), moisture analyzer (OHAUS®), freeze dryer (BIOBASE®).

Methods

Determination of plant

Kirinyuh and mareme plants were determined at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran.

Preparation of kirinyuh and mareme leaves

The leaves are selected and separated from damaged, rotten, and black leaves. The leaves are washed using clean running water, allow to dry from the washing water, then the leaves are cut to reduce the size to make it easier for the blending process. The juice from the blended leaves was filtered using a filter cloth. The juice is then dried using a Freeze dryer [7].

Phytochemical screening

Phytochemical screening of kirinyuh and mareme leaves, including alkaloids, tannins, flavonoids, triterpenoids/steroids, and saponins were carried out [8].

Preparation of effervescent granules from kirinyuh and mareme leaves

The formula for effervescent granules from kirinyuh and mareme leaves were shown in table 1. The preparation of effervescent granules was carried out using the wet granulation method. Preparation of the preparation begins with preparing the materials and tools that will be needed, and weighing all the ingredients that will be used according to

the needs of each formula. The manufacture of acid and base granules is carried out separately. Mix the dried kirinyuh leaf and mareme juice from each formula and grind until smooth, then set aside. The basic part of the granule consists of sodium bicarbonate, some lactose, and part of the dry leaf extract mixture, which is finely ground until homogeneous, while the acidic part consists of citric acid, the remaining dry leaf extract mixture, and the remaining lactose which is finely ground until homogeneous. The acid and base parts were each moistened with PVP K-30, which had been dissolved using ethanol and added melon essence to form granules. The acid and base phases were sieved using mesh no. 14, then dried using an oven at a temperature of 48 ^{0}C for±6 h until the granules are dry; after they are dry and cool, then the granules are sieved using mesh no. 16, and added aerosil until homogeneous [8].

Table 1: Effervescent granule formula

Materials	F I (%)	F II (%)	F III (%)
Kirinyuh Leaf Extract	1	2	3
Mareme Leaf Extract	1	2	3
Sodium Bicarbonate	30	30	30
Citric Acid	21	21	21
Aerosil	0.5	0.5	0.5
PVP K-30	4	4	4
Melon *Essence*	q. s	q. s	q. s
Lactose ad	100	100	100

Source: Gustaman *et al.*, 2021.

Evaluation of effervescent granules

Organoleptic test

Observing the granule dosage form made, including an assessment of the odor, color, shape and taste of the effervescent granule preparation made [8].

Test of flow time and angle of repose

A total of 50 g of effervescent granules were poured little by little into a flow tester that had the end closed, the funnel lid was opened at the same time as the stopwatch was started, recording the time it took for the granules to flow [9]. Take measurements on the granules formed using the formula:

$$\tan \alpha = \frac{\text{height (h)}}{\text{radius (r)}}$$

Water content determination

A total of ±5 g of effervescent granules were added to the moisture analyzer. Measure the water content contained in the prepared granules. The requirement for effervescent water content is less than 5% [10].

Dissolving time test

A total of 10 g of effervescent granules are put into a glass, then add 200 ml of water. The dissolving time of good effervescent granules is less than 5 min [11].

pH test solution

A total of 10 g of effervescent granules are put into a glass, then add 200 ml of water. Then check the pH using a pH meter. The result of a good pH of the effervescent solution is when the pH value is close to neutral [11].

Compressibility test

A total of 48 g of granules were put into a 100 ml measuring cup, then recorded the height of the granules as V0 (mL). The measuring cup containing granules is stored in the tap density tester and knocking until the volume does not decrease anymore (±500 taps) is recorded as Vt [12]. Calculate the compressibility of the granules made using the formula:

$$\text{Tapped density} = \frac{\text{granule weight}}{\text{Vt}}$$

$$\text{Bulk density} = \frac{\text{granule weight}}{\text{V0}}$$

$$\text{Carr}^{'}\text{s index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \text{x } 100\%$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

Antioxidant activity test

DPPH solution preparation

DPPH was weighed as much as 50 mg then dissolved with methanol in a 50 ml volumetric flask to obtain a solution with a concentration of 1000 ppm, take some of the solution to be diluted to obtain a concentration of 30 ppm DPPH solution.

DPPH solution absorption measurement

Measure the absorption spectrum of 30 ppm DPPH solution using a UV-Vis spectrophotometer at a wavelength of 510-520 nm, then determine the maximum wavelength.

Setting operating time

Determination of operating time is done by using DPPH solution with mother liquor. Vitamin C was measured with the maximum wavelength previously obtained; then the absorbance was measured every two minutes for one hour. The minute that produces a constant absorbance is the operating time.

Vitamin C preparation

Weigh as much as 50 mg of vitamin C, then do the dissolving of vitamin C with methanol in a 50 ml volumetric flask until a solution with a concentration of 1000 ppm is obtained.

Antioxidant activity t of vitamin C

The mother liquor of vitamin C was diluted to obtain concentrations of 1, 2, 3, 4 and 5 ppm. A total of each solution was taken using a micropipette and added with 2 ml of DPPH solution with a concentration of 30 ppm. Measure the absorbance at a wavelength of 516 nm.

Antioxidant activity test of effervescent granules

The sample of each formula was weighed as much as 50 mg; then the sample was dissolved using methanol in a 50 ml volumetric flask which then obtained a solution with a concentration of 1000 ppm. The solution was then diluted with concentrations of 200, 400, 600, 800, and 1000 ppm. A total of each dilution solution was taken and added with 2 ml of DPPH solution with a concentration of 30 ppm using a micropipette. Then let stand for 30 min to incubate. After that, measure the absorbance at the maximum wavelength of DPPH.

Measurement of antioxidant activity

Measurement of the antioxidant activity content of effervescent granule samples from kirinyuh leaves and mareme leaves using the formula:

$$\% \text{ inhibition} = \frac{\text{A blanko} - \text{A sample}}{\text{A blanko}} \text{ x } 100\%$$

From the resultant value of % inhibition, IC_{50} was determined [13]. By using the formula:

$$IC_{50} = \frac{50 - a}{b}$$

The values of α and b are obtained from the linear regression equation, y = bx+α.

RESULTS

The results of the determination of the plant through the plant identification sheet number 12/HB/01/2022 that the plant used is true kirinyuh leaf with the scientific name *Chromolaena odorata* (L.) R. M. King and H. Rob and plant identification sheet number 13/HB/01/2022 that the plant is actually a mareme plant with the scientific name *Glochidion arborescens* Blume. Determination is carried out, which aims to ensure the truth and clarity of the plants to be used in the study so as to avoid errors in the use or sampling to be used. A total of 1.2 liters of kirinyuh leaf extract, which was then freeze-dried obtained dry powder weighing 39.09 g. As for the mareme leaves as much as 1.5 liters of leaf extract obtained dry juice weighing 47.20 g. Phytochemical screening is a type of preliminary test to determine the content of secondary metabolites that can be used as active substances that can have activities contained in leaf extract samples. The results of phytochemical screening can be seen in table 2.

Table 2: Phytochemical screening results

Compounds	Kirinyuh	Mareme
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Quinones	-	+
Triterpenoids	+	+

Description: (+) detected, (-) not detected

The results of the screening test for kirinyuh leaf extract and mareme leaf extract contained alkaloids; when the extract was added with Dagendroff reagent to produce a precipitate, the precipitate was orange in color. And when Wagner's reagent is added, a brown precipitate is formed. These results indicate that the extracts of the two leaves contain alkaloids [14]. Screening for flavonoids, kirinyuh leaf extract and mareme leaves showed positive for containing flavonoids because they produced a yellow color in the amyl alcohol layer [15]. Furthermore, screening for saponins, kirinyuh leaf extract and mareme leaves showed positive for saponins because they produced stable foam [16]. Screening for tannins, kirinyuh leaf extract and mareme leaves showed positive tannin content because a black color was formed in the juice [17]. Screening for quinones, kirinyuh leaf extract was negative for quinone while mareme leaf extract showed positive for quinone because a red color was formed in the juice [15], and the last test was screening for triterpenoid content, kirinyuh leaf extract and mareme leaf showed positive content of triterpenoids because they produced a ring orange [16].

The results of the preparation of effervescent granules using dried extracts of kirinyuh leaves and mareme leaves with a combination of leaves 1: 1. Formula 1 concentration of 1% each leaf, Formula 2 concentration of 2% each leaf, and in formula 3 with a concentration of 3% each leaf. Can be seen in fig. 1.

The results of the color examination of the granule preparations that were made to produce color in formula 1 produced pale green granules, formula 2 produced green granules, and formula 3 produced dark green granules. The results of organoleptic tests can be seen in table 3. The flow time test for the effervescent granule preparations can be seen in table 4. The test results obtained from testing the moisture content of the effervescent granule preparations from kirinyuh leaves and mareme leaves can be seen in table 5. The results of the dissolving time of the preparation can be seen in table 6. The results of the pH test can be seen in table 7.

(a) **(b)** **(c)**

Fig. 1: Granule (a) formula 1, (b) Formula 2, (c) Formula 3

Table 3: Organoleptic of effervescent granule

Testing	Result		
	F I	F II	F II
Form	Granule	Granule	Granule
Color	Pale Gren	Light Green	Dark Green
Scent	Weak Aromatic	Aromatic	Strong Aromatic
Flavor	Sour	Sour	Sour

Table 4: Flow time and angle of repose (n = 3)

Formula	Flow time (sec)	Angle of repose (^{0})
1	04.61±0.276	33.78±1.209
2	04.88±0.035	35.11±1.114
3	04.61±0.658	35.68±1.314
Requirement	50 g/5 sec	25^0–45^0

Table 5: Water content (n = 3)

Formula	Water content (%)	Requirement (%)
1	2.09±0.000	
2	2.59±0.006	<5%
3	2.37±0.006	

Table 6: Dissolving time (n = 3)

Formula	Dissolving time (min)	Requirement
1	01.49±0.031	<5 min
2	02.12±0.266	
3	01.36±0.015	

Table 7: Granule pH (n = 3)

Formula	pH	Requirement
1	6.57±0.000	
2	6.44±0.733	6–7
3	6.10±0.404	

The results of the compressibility test and the value of Hausner's ratio on the effervescent granule preparations from kirinyuh leaves and mareme leaves can be seen in table 8. which is less than 15%. Meanwhile, the value of Hausner's ratio can be said to be good if it is in the range of 1.00-1.18 [12].

Table 8: Compressibility test result (n = 3)

Formula	*Hausnes's ratio*	Compressibility (%)
1	1.064±0.00	6.06±0.00
2	1.075±0.00	6.98±0.00
3	1.064±0.00	6.06±0.00
Requirement	1.00–1.18	<15%

Antioxidant testing of the effervescent granule preparations from kirinyuh leaves and mareme leaves was carried out to determine the antioxidant activity of the preparations made using the DPPH method. The DPPH method was chosen because it is a fast, simple antioxidant testing technique or method, and does not use many chemical reagents. The use of the DPPH method also requires only a small number of samples [18]. The IC_{50} value is the concentration value that results in 50% loss of DPPH activity. A sample can be said to contain very strong antioxidant activity if it is in the range<50 ppm, and has very weak antioxidant activity if it has an IC_{50} value of>200 ppm [19].

Furthermore, the determination of the maximum wavelength of DPPH is carried out. Measurement of the maximum wavelength of DPPH with a concentration of 30 ppm is at a wavelength of 516 nm. The wavelength is used to measure the level of antioxidant activity contained in all effervescent granule formulations that are made. In the antioxidant activity test using the DPPH method, there is a method for determining the operating time. This measurement is used to determine the length of time required to achieve a stable absorbance value [20]. In testing the operating time is carried out every 2 min for 1 hour, and the operating time is obtained at the 25 min.

Then measured the antioxidant activity of vitamin C with 30 ppm DPPH solution. The mother liquor of vitamin C with a concentration of 1000 ppm was diluted with concentrations of 1, 2, 3, 4 and 5 ppm to determine the concentration of antioxidant activity. Then from the concentration obtained the results of the linear regression equation of vitamin C with DPPH solution can be seen in fig. 2.

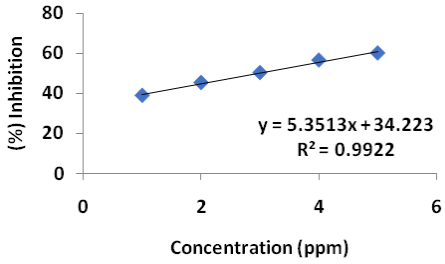

Fig. 2: Vitamin C antioxidant activity curve

From the test results, the IC_{50} value of vitamin C was 2,948 ppm, with the category including having very strong antioxidant activity. The test was repeated one time because it was related to the problem of time and sample availability. Vitamin C is used as a comparison solution because vitamin C is a type of secondary antioxidant that has the ability to capture free radicals [4].

Then, further testing of the antioxidant activity contained in the effervescent granule preparations from kirinyuh leaves and mareme leaves using UV-Vis spectrophotometry was measured with the obtained wavelength at 516 nm. Each formula contains different concentrations of active substances, for formula 1 contains kirinyuh leaf and mareme leaf each 1%, formula 2 contains kirinyuh leaf and mareme leaf each 2%, and formula 3 contains kirinyuh leaf and mareme leaf each 3%. The IC_{50} values for the three effervescent granule formulations from kirinyuh and mareme leaves can be seen in table 9.

Table 9: Sample and standard IC_{50} (n = 1)

Sample	IC_{50} value (ppm)	IC_{50} standard (ppm)
Formula 1	946.55	
Formula 2	720.80	2.948
Formula 3	581.67	

Based on the results of the antioxidant activity test of the effervescent granule preparations from kirinyuh and mareme leaves, the IC_{50} values for formula 1, formula 2, and formula 3 were 946.55 ppm, 720.80 ppm, and 581.67 ppm, respectively. Due to time and sample availability, each effervescent granule formula was tested once. According to Riwanti (2021) that a substance or sample still has antioxidant activity properties if the IC_{50} value obtained is still in the range of 200-1000 ppm, where the substance or sample is less active but still has potential activity as an antioxidant substance [21].

DISCUSSION

Extracts from kirinyuh leaves and mareme leaves were dried using a freeze dryer. Freeze dryer is a technique to dry a substance or material. The mechanism of the freeze dryer technique is that it begins with the freezing process of the sample, which is then dried by separating or removing most of the water content contained in the sample by means of a sublimation process. The drying process occurs when the vacuum is at a very low temperature and takes place when the sample is frozen and then the water is removed, which was initially in the form of ice or frozen to water vapor without going through a liquid phase process [22]. The advantages of using the freeze dryer method are the fast drying rate process, in terms of the quality of the freeze dryer results do not cause wrinkles on the surface of the sample, the sample is easily refreshed, the color of the sample remains normal, and the nutritional value or active substance of the sample can be maintained [23]. The resulting color is different due to differences in the concentration of different leaf; the higher the concentration of leaves, the darker the color of the granules. For the taste and shape of the granules, a sour taste is obtained, and the granules are uniform. For the aromas of formulas 1, 2, and 3, a very weak melon aroma is produced, but the aroma formed is more leafy, where the higher the concentration of leaves in each formula, the stronger the aroma of the leaves.

Granule flow time is a test to determine the time required for granules to flow; this granule time test aims to determine the ability of the granule preparations made to be filled into granule packaging. The granule flow time can be said to be good if as much as 50 g of granules flow within 5 seconds [5]. Furthermore, test the angle of repose of the granules, the angle of repose is the angle formed from the granules as a result of testing the flow time. The granule angle of repose test is carried out by measuring the height of the effervescent granule pile and measuring the base or base radius of the effervescent granule pile. Based on the test, the results of the angle test for each formula can be seen in table 4. The results of a good granule angle of repose are in the range of 25 -45^0 [24]. This is confirmed by Dyera's research, that the results of the angle of repose of the granules with a range of 31^0–35^0 are said to be good [11].

The test results obtained from the preparation of effervescent granules made that the granules have a good angle of repose value and meet the requirements. The granule moisture content test was carried out to determine the water content contained in the granule preparations made. If the water content exceeds the requirements specified in the granule preparation, it can trigger a reaction from the effervescent granule preparation before dissolving, and if the effervescent granule does not meet the water content requirement, it will cause the granule to have poor flowability. The requirements for the water content of granule preparations are 2–4% [5], while according to BPOM number 12 of 2014 the water content for effervescent is less than 5% [10]. The solubility test of effervescent granules was carried out to determine the required dissolving time of the prepared preparation; the soluble preparation was marked when the foam or carbon dioxide gas produced in the water stopped. The dissolving time of the granule preparation is related to the water content or humidity of the granule preparation, the lower the water content in the granule preparation, the granules will dissolve quickly [12]. Stirring can affect the dissolving time, the time required for the three formulations of the effervescent granule preparations from kirinyuh leaves and mareme leaves extract that are made to meet the dissolving time requirements, where the dissolving time of good effervescent granules is less than 5 min [11].

The pH test was carried out on the effervescent granule preparations that were made to determine the degree of acidity of the prepared preparations. It is recommended to measure pH because it is to find out whether the effervescent granule preparation made meets the requirements or not. If the effervescent solution has a pH that is too alkaline it will cause an unpleasant and bitter taste, whereas if the effervescent solution is too acidic it can cause gastric irritation [12]. There is a difference in the pH value of each formula, where the greater the concentration of leaves added, the pH also decreased. Of each formula tested, each formula has a good pH value and meets the requirements. The result of a good pH of the effervescent solution is when the pH value is close to neutral [11].

The compressibility test was carried out to determine the flowability of the granules made so that the percent (%) compressibility value was obtained. The size of the granules in each formula can affect the results of compressibility; the particle size of the small or finer granules will fill the gaps or cavities in the large granules, causing the volume of the effervescent granule preparation to shrink. The results of the compressibility test and the value of Hausner's ratio on the effervescent granule preparations from kirinyuh leaves and mareme leaves is less than 15%. Meanwhile, the value of Hausner's ratio can be said to be good if it is in the range of 1.00-1.18 [12].

From the results of the IC_{50} value, it shows that the smallest IC_{50} value among the three formulas is found in formula 3, which is 581.667 ppm. Then an experiment was also conducted to test the antioxidant activity of the dosage formula containing the dried extract of kirinyuh leaves and mareme leaves each with a concentration of 20%; the IC_{50} value was 219.629 ppm. It can be compared from the results of the IC_{50} value that each formula containing high concentrations of kirinyuh leaves and mareme leaves will get better antioxidant activity results which can be seen from the IC_{50} value.

The results of the antioxidant activity of all the formulas made have IC_{50} values that are very far from the results of the IC_{50} values of the tested vitamin C, each effervescent granule formulation has very weak antioxidant activity results when compared to the results of antioxidant activity of vitamin C which has very weak antioxidant activity. This could be due to the fact that the antioxidants contained in the formula are not pure compound forms but have been mixed with the ingredients in the effervescent granule formulations and have gone through long processes in the manufacture of granules that affect the antioxidant activity in the dosage form.

CONCLUSION

Extracts from kirinyuh leaves (*Chromolaena odorata* L.) and mareme leaf extracts (*Glochidion arborescens* Blume) can be formulated as effervescent granules. The evaluation results of the three formulas met the requirements of the physical properties of effervescent granules. The three formulations of effervescent granule preparations from kirinyuh leaves and mareme leaves have very weak antioxidant activity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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