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Research Article

EVALUATING OF EFFERVESCENT TABLETS CONTAINING GRAPE SEED (*VITIS VINIFERA* L.) EXTRACT AS A NUTRACEUTICAL

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ABSTRACT

Objective: Grape is one of the most well-known fruits. People usually consume only the fruit and the skin; however, the seed is the part of the fruit that contains important antioxidant rich polyphenol. However, grape seed and its extract have an unpleasant taste. Therefore, this study aimed to formulate effervescent tablets containing grape seed extract (GSE) to overcome the unpleasant taste.

Methods: Effervescent tablets of GSE were formulated using three formulas, each with a different percentage of the effervescent mix. The tablets were prepared using wet granulation method at 40% relative humidity (RH) (the ratio of the partial pressure of water vapor to the equilibrium vapor pressure of water) and 25°C temperature. The effervescent granules and the tablets were evaluated for various characteristics in term of granules flowability, moisture content, as well as tablets appearance, size and weight uniformity, hardness, friability, effervescence time, pH, and total phenol content. In addition, all three formulations of the effervescent tablets and solutions were evaluated for appearance, taste, and flavor using the hedonic test that involved 30 panelists.

Results: The evaluation of the effervescent granules and tablets showed that they had good characteristics. The disintegration time of the three formulations was within the acceptable range, between 3.67 minutes and 4.69 minutes. The pH of the effervescent solution was between 5.18 and 5.80. Based on the hedonic test, all the effervescent solutions had favorable appearance, taste, and flavor.

Conclusions: Clinical *Streptococcus salivarius* isolates from the dorsum of the tongue had greater potential for inhibiting *Enterococcus faecalis* growth compared to the saliva isolates and control bacteria. Therefore, we can conclude that the effervescent tablets containing grape seed extract are potential be used as a nutraceutical dosage form.

Keywords: Effervescent tablets, Nutraceutical, Grape seed

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INTRODUCTION

Nowadays, people are increasingly concerned about their health, therefore seeking methods to preserve their health; one of these methods is the consumption of nutraceuticals. The term "nutraceutical," a combination of the words "nutrition" and "pharmaceutical," was first introduced in 1989 by Stephen DeFelice, MD, a researcher and the chairperson of the Foundation for Innovation in Medicine. According to DeFelice, a nutraceutical is a food (or a part of food) that provides medical or health benefits, including the prevention and/or treatment of diseases [1]. Grape is a fruit with many health benefits. Particularly, the seeds of grapes have many polyphenols that function as antioxidants and anticancer agents as well as provide cardioprotection [2]. A previous study states that a dose of 300 mg/day of grape seed extract (GSE) can lower blood pressure levels in patients with prehypertension [3]. Considering the benefits of GSE, it can be used to formulate an innovative nutraceutical product such as effervescent tablets.

Effervescent tablets were chosen because they can be used to make a solution with a good flavor, masking the bitterness of the GSE. The bubbling caused by the presence of carbonate can improve the taste of some active ingredients in the effervescent tablets [4]. When placed in water, the effervescent tablets rapidly form a clear solution [4]. In this study, GSE effervescent tablets were prepared using wet granulation method with different concentrations of the effervescent mix: 50% for Formula 1 (F1), 60% for Formula 2 (F2), and 70% for Formula 3 (F3). The effervescent granules were evaluated for several parameters such as flow rate, compressibility index, Hausner's ratio, angle of repose, and moisture of the granules. The effervescent tablets were evaluated for weight and size uniformity, hardness, friability, dissolution time, and pH of the solution. In addition, all three formulations of the tablets and the effervescent solution of the GSE were tested by the panelist for appearance, flavor, and taste.

MATERIALS AND METHODS

Materials

GSE (Sciyu Biotech Co., China), citric acid (Budi Starch, Indonesia), tartaric acid (Legre Mante, Perancis), sodium bicarbonate (Natural Soda, America), mannitol, maltodextrin, polivinil pirolidon K-30 (BASF, America), polyethylene glycol (PEG) 4000, aspartame (Ajinomoto, Japan), blackcurrant flavor (Taiwan), Folin–Ciocalteu (Merck, Germany), gallic acid (Sigma, America), sodium carbonate (Merck, Germany), alcohol 96% (Acidatama, Indonesia), and aquadest (Brataco, Indonesia).

Preparation of the effervescent tablets

The tablets were prepared in a room with 40% relative humidity (RH) and 25°C temperature. The granulation process was performed in the following three steps: (1) The production of acid and alkali granules, (2) the addition of the lubricant, and (3) the preparation of the tablets using a machine [5]. The acid granules comprised citric acid, tartaric acid, aspartame, mannitol, maltodextrin, GSE, blackcurrant flavor, and purple dye. The alkaline granules contained sodium bicarbonate, mannitol, maltodextrin, GSE, blackcurrant flavor, and purple dye. Both types of granules were mixed with polyvinylpyrrolidone in alcohol 96% for binding. Thereafter, the mixtures were passed through an 8-mesh screen and dried in an oven at 50°C for 8 hrs. Then, the acid and alkaline granules were passed through a 16-mesh screen and mixed homogeneously. Finally, PEG 4000 was added as a lubricant and mixed. The formulations of the GSE effervescent tablets are presented in Table 1.

Table 1: Formulations of the GSE effervescent tablet

Materials	F1		F2		F3	
	(%)	(g)	(%)	(g)	(%)	(g)
GSE	1.11	0.0500	1.11	0.0500	1.11	0.0500
Effervescent mix						
Citric acid	8.5	0.3825	10.2	0.4590	11.9	0.5355
Tartaric acid	15.5	0.6975	18.7	0.8370	21.7	0.9765
Sodium bicarbonate	26	1.1700	31.2	1.4040	36.4	1.6380
Mannitol	25.82	1.1620	15.82	0.7120	5.82	0.2620
Maltodextrin	11	0.4950	11	0.4950	11	0.4950
PEG 4000	4	0.1800	4	0.1800	4	0.1800
PVP	1	0.0450	1	0.0450	1	0.0450
Aspartame	2	0.0900	2	0.0900	2	0.0900
Blackcurrant flavor	5	0.2250	5	0.2250	5	0.2250
Violet dye	0.07	0.0030	0.07	0.0030	0.07	0.0030
Total	100	4.5000	100	4.5000	100	4.5000

GSE: Grape seed extract, PEG: Polyethylene glycol, PVP: Polyvinylpyrrolidone

Evaluation of the effervescent granules

Flowability

Flowability of the granule mass was determined using the angle of repose, Hausner's ratio, and the compressibility index.

Angle of repose

The powder or the granule mass *was passed* through a funnel. The angle of repose was determined using equation 1.

$$\alpha = \text{Arc}\tan(\frac{h}{r}) \quad (1)$$

Hausner's ratio and compressibility index

The evaluation of these was conducted using a tap bulk density tester and determined using equations 2 and 3.

$$\text{Hausner's ratio} = \frac{\rho \text{ tapped}}{\rho \text{ bulk}} \quad (2)$$

$$\text{Compressibility index} = \frac{(\rho \text{ tapped} - \rho \text{ bulk})}{\rho \text{ tapped}} \times 100\% \quad (3)$$

Moisture content

The moisture content of the granules was determined using a moisture balance. It shows the moisture value of the granule after the water level reaches a constant value.

Evaluation of the effervescent tablets

Appearance of the effervescent tablets and solution

The overall appearance, including the shape, color, and state of the tablet surface as well as the color and clarity of the effervescent solution was rated [6].

Size uniformity

The thickness and diameter often tablets were measured using calipers. The diameter of the effervescent tablets ranged from 2.52 cm to 2.54 cm [7].

Weight uniformity

Ten tablets were selected randomly and weighed; thereafter, their average weight was calculated [7].

Hardness test

The hardness often tablets of each formulation was determined using a hardness tester [8].

Friability test

Twenty tablets of each formulation were selected randomly, and after measuring their total weight, they were placed in the friabilator chamber (Erweka, TAP, Germany) for 4 minutes at 25 rpm. Tablets for which the weight loss was lower than 1% passed the friability test [7].

Effervescence time

A single tablet was placed in a beaker containing 200 mL of aquadest at 25°C. Whenever a clear solution without particles was obtained; the effervescence was considered complete. The mean value of the three measurements for each formulation was reported [9].

pH of the effervescent solution

One tablet was allowed to dissolve in 200 mL of purified water at 20 ± 1°C; the pH was then determined using a pH meter immediately after the tablet had completely dissolved. This experiment was repeated 3 times for each formulation [10].

Total phenol content in GSE

The evaluation of the total phenol content was performed using the Folin–Ciocalteu method. The total phenol content was calculated using a standard calibration curve of gallic acid [11].

Total phenol content of the effervescent tablets of GSE

Twenty tablets were ground until smooth and homogeneous [8]. Thereafter, the total phenol content was determined using the Folin–Ciocalteu method and calculated using a calibration curve equation of gallic acid as the standard [11].

Hedonic test

The hedonic test was conducted for each formulation of the effervescent tablets and solution of GSE by 30 untrained panelists (Table 2). Each panelist was asked to fill out questionnaires that required him/her to rate the flavor, aroma, and appearance. The responses included the following levels of preference: Like very much, like, neither like nor dislike, dislike, and dislike very much. Results of the assessment were tested statistically using SPSS [10].

RESULTS AND DISCUSSION

Preparation of the effervescent tablets

The effervescent tablets were prepared using wet granulation method. During the granulation process, acid, and alkaline granules were separated to avoid an early effervescent reaction. In addition, the process was performed in a room with a relative humidity (RH) of 40% and a temperature of 25°C. This formulation was prepared using a combination of the two acids to produce granules with desirable characteristics. If only citric acid is used, the mixture will become stickier and difficult to granulate. However, if we use only tartaric acid, the granule strength will be lower. Therefore, in this study, we used a combination of citric acid and tartaric acid [5]. Three formulations of the GSE effervescent tablets were prepared with different concentrations of the effervescent mix: 50%, 60%, and 70%, and all three were evaluated to determine the differences in their tastes and flavor.

Granules flow rate
The granules flow rates of three formulations were good (>10 g/s) as they were sifted twice to ensure uniformity in the granule sizes. In the process of tablet compressions, the granules flow rate is an important factor, since it will affect the tablet weight uniformity as one of the requirements of tablet quality.

Angle of repose
The angles of repose of the three formulations were excellent, as indicated by the resulting break angle that ranged from 27.31° to 28.46°, which was within the acceptable range of 25°-30°. The value of the three formulations was not very different; this may be attributable to the fact that the granules were uniform in size.

Hausner's ratio and compressibility index
The Hausner's ratios of the three formulations were between 1.13 and 1.17, indicating that they had good flowability properties. In addition, the compressibility index test was used to determine the flow properties of the granules that enable them to form a stable and compact mass at a given pressure. The values of the compressibility indices (11.82-14.38%) of the three formulations indicated good flowability properties. The smaller the value of the compressibility index, the greater is the flowability of the granules [4].

Moisture content
The moisture contents of the three formulations were quite low (1.24-1.40%). The low moisture content values of the granules were expected to prevent early effervescent reaction and sticking of the tablets during the manufacturing process (Table 3).

Appearance of the GSE effervescent tablets
Physically, the GSE effervescent tablets had a flat, round shape and a smooth surface without any chipped portions. Its color was purplish gray with dark purple spots owing to the addition of purple dye into the formulation. The addition of acids caused some granules to become darker than the granule base, resulting in dark purple spots on the tablet. In addition, the effervescent tablet had a sour taste and no smell.

Appearance of the GSE effervescent solution
The physical appearance of the GSE effervescent solution was dark purple and not very clear. In addition, the effervescent solution formed slight foam. The turbidity and the appearance of the foam may be due to the interaction between the extract and the excipients because the effervescent tablets without the extract solution were clear.

Size uniformity
The diameter of the tablets of each formulation was 2.52 cm. These results are in accordance with the standard, recommended a diameter of the effervescent tablets (2.52 cm to 2.54 cm) 11. The effervescent tablet formulations had different thicknesses; this may be due to the differences in the pressure exerted at the time of tablet production.

Weight uniformity
The average weights of the effervescent tablets of the three formulations were 4502.9 mg, 4508.1 mg, and 4501.9 mg for F1, F2, and F3, respectively. All the formulations met the requirements of the coefficient of variation (<6%) (Table 4) [7].

Hardness test
The effervescent tablet had the requisite hardness (>10 Kp) [4]. This also used as a method of physical control over the manufacture [4]. Formula 1 generated hardness 20.01 Kp, formula 2 at 25.26, while formula 3 produce the highest hardness (29.94 Kp). The variation in the hardness values may have been caused by the addition of a binder. In addition, the maltodextrin may also have acted as a binder [12], increasing the hardness of the tablet.

Friability test
Friability was also calculated to determine the strength of the tablets produced (Table 5). The friability test was used to determine the resistance of the tablet to shocks that occur during the manufacturing process, packaging, and distribution. All three formulations passed the friability test (value <1%) 6.

Effervescence time
The F3 effervescent tablets showed the fastest effervescence time followed by F2 and F1 as per the data in Table 6. The F3 effervescent tablets were formulated with a high concentration of the effervescent mix (70%). It indicated that the higher the concentration of the effervescent mix, the faster the effervescence time. This can be explained by the fact that a higher concentration of the effervescent mix produces more amount of carbon dioxide (CO_2).

pH
The pH values of the effervescent tablet solutions were 5.18 (F1), 5.44 (F2), and 5.80 (F3). Variations in the pH values may be due to the different concentrations of the effervescent mix. However, the measured pH values for all effervescent tablet solutions were within the expected range of pH values (5-6). A solution with a pH range of 5-6 is not too acidic. Therefore, the effervescent preparation was found to be safe for consumption. In addition, the slightly acidic nature can provide a fresh taste when consumed.

Table 2: Numeric and descriptive scale for hedonic test

Numeric scale	Descriptive scale
5	Like very much
4	Like
3	Neither like nor dislike
2	Dislike
1	Dislike very much

Table 3: Various parameters of the effervescent granules

Parameters	Results		
	F1	F2	F3
Flow rate (g/s)	11.00±0.49	10.56±0.67	11.53±0.22
Angle of repose (°)	27.31±1.49	28.46±1.05	28.23±1.45
Hausner's ratio	1.17±0.00	1.16±0.02	1.13±0.01
Compressibility index (%)	14.38±0.36	13.88±1.17	11.82±0.52
Moisture content (%)	1.37±0.20	1.40±0.10	1.24±0.20

Table 4: Size and weight uniformity of the GSE effervescent tablets

Parameter	Tablet F1	Tablet F2	Tablet F3
Diameter (cm)	2.52±0.00	2.52±0.00	2.52±0.00
Thickness (cm)	0.68±0.00	0.65±0.01	0.64±0.01
Weigh (mg)	4502.9±0.69	4508.1±0.93	4501.9±0.46

GSE: Grape seed extract

Table 5: Evalution of the hardness and friability tablets

Parameter	Tablet F1	Tablet F2	Tablet F3
Hardness (Kp)	20.01±0.93	25.26±0.47	29.94±0.39
Friability (%)	0.87	0.73	0.64

Table 6: Evalutions of the effervescence time and pH of the effervescent tablet solutions

Parameter	F1	F2	F3
Effervescence time (minute)	4.69±0.17	3.81±0.03	3.67±0.04
pH of the effervescence solution	5.18±0.03	5.44±0.03	5.80±0.03

Table 7: Total phenol content of the GSE effervescent tablets

Formula	Total Phenol content (mg GAE/g)
1	11.52±0.19
2	11.76±0.25
3	12.30±0.96

GAE: Gallic acid equivalent, GSE: Grape seed extract

Total phenol content

The total phenol content of the GSE effervescent tablet was lower than that of the extract alone (Table 7). GSE had a total phenol content of 25.35±0.90 mg gallic acid equivalent (GAE)/g extract, while the total phenol content of the formulations ranged from 11.52 to 12.30 mg GAE/g extract. A low total phenolic content in the granules than in the extract itself can be due to the drying process during granulation. The total phenolic content is used as a marker of phenolic compounds useful as antioxidants in GSE. Phenolic compounds such as proanthocyanidins, catechins, and epigallocatechin are present in GSEs [13]. A previous research has stated that the total phenol content of different varieties of grape seeds will be different [12].

Hedonic study

The hedonic study was analyzed based on the scales of the effervescent tablet appearance as well as the effervescent solution appearance, flavor, and aroma using the Kruskal-Wallis test. The results showed that all GSE effervescent tablets of three different formulas were not significantly different, based on the $p>0.05$ for their appearance, taste, and aroma. The appearance of the effervescent tablets and the effervescent solution is very important to attract the interest of consumers. Based on the results of about 80% of the panelists, most liked the appearance of the F3 effervescent tablets. During their interviews, the panelists said that the appearance of the tablets (in terms of the purple color) was good; however, they said that the purple spots made the tablet less attractive.

Of the 80% panelists who were asked about the appearance of the F3 effervescent tablet solution of GSE, about 20% liked it. In the opinion of the panelists, the purple color of the solution indicated that they contained grape. Therefore, the appearance of the GSE was reported to be preferable. The panelists reported that they liked the appearance of the GSE effervescent tablets and the effervescent solution that represented the color of grapes.

The hedonic study regarding the taste of the three GSE effervescent solutions showed no significant differences. This may have been because all three tablet formulations contained the same proportion of sweetener. However, the highest percentage of panelists (43.33%) choose "neither like nor dislike" for F3. In addition, in the interviews, the respondents said that the flavor of F3 was too acidic. Therefore, the panelists who do not like sour taste did not like F3. F1 had a sweeter taste because the concentration of effervescent used for it was lower than that in F2 and F3; moreover, the percentage of mannitol was higher in F1. However, all three formulations were rated "acceptable" by the panelists. From the results of the test regarding the taste of the effervescent tablet solutions, we can conclude that the concentration of the effervescent mix used affects the taste of the effervescent solution. Therefore, the appropriate concentration of the effervescent mix should be identified to make it more acceptable to the panelists.

The third parameter of the test was flavor. Flavor is also an important factor that influences the consumer acceptability. The flavor of the GSE effervescent tablets was blackcurrant. The blackcurrant flavor was chosen because it represents the purple color of the effervescent solution. Use of the right flavor concentration is important to enable it to mask the smell and taste of GSE. The results indicated that about 80% of the panelists found the flavor of all three formulations of the GSE effervescent solution preferable. Thereby, we can conclude that the panelists liked the flavor of all three GSE effervescent tablet solutions.

CONCLUSION

According to the evaluation results of the granules and the effervescent tablets, all produced effervescent tablets (F1, F2, and F3) met the requirements of an effervescent tablet. In addition, most of the panelists found all the GSE effervescent tablets, which were formulated from three kind's formulations, were acceptable in terms of appearance, taste, and aroma. Therefore, it can be concluded that the GSE effervescent tablets have the potential to be used as a nutraceutical product.

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