

# Potential Granule Effervescent from a mixture of beetroot extract (*Beta vulgaris* L), Malang apple (*Malus sylvestris* L.) and Carrot (*Daucus carota* L.) as Preparation for Lowering Blood Cholesterol Levels

Ratnaningsih DA  
Palembang Health Polytechnic  
Pharmacy Department  
Indonesia

Sarmalina Simamora  
Palembang Health Polytechnic  
Pharmacy Department  
Indonesia

Tedi  
Palembang Health Polytechnic  
Pharmacy Department  
Indonesia

Muhamad Taswin  
Palembang Health Polytechnic  
Pharmacy Department  
Indonesia

**Abstract:-** This study aims to test the potency of granule effervescent from a mixture of beetroot (*Beta vulgaris* L.), Malang apple (*Malus sylvestris* L.) and carrot (*Daucus carota* L.) extracts as preparations for lowering blood cholesterol levels. In this study, beetroot, apple and carrot were used as aqueous extracts and made into 8 effervescent granule formulations. 4 formulas with variations of citric acid, tartaric acid and sodium bicarbonate 1:1:1 and 4 formulas with a ratio of 1:2:3. Each formula with acid-base variations will be added with a mixture of beetroot, apple and carrot extract (1:1:1) with levels of 15%, 20% and 25% and one formula without extract as a control. After knowing its stability, the preparation was tested for its potential as cholesterol-lowering in 9 groups of male Wistar white rats, each group consisting of 4 rats given a high-cholesterol diet for 14 days. Cholesterol levels were checked on the 0, 7 and 14 days using a digital cholesterol checker. The study results were analyzed using one-way ANOVA statistical test ( $p < 0.05$ ). From the test results on rats, it was found that all effervescent granule formulas containing mixed extracts of beetroot, apple and carrot extract (1:1:1) with levels of 15%, 20% and 25% could reduce blood cholesterol levels in mice. It can be concluded that granule effervescent from a mixture of aqueous extracts of beetroot (*Beta vulgaris* L), Malang apple (*Malus sylvestris* L.) and carrot (*Daucus carota* L.) (1:1:1) can be used as preparations for lowering total cholesterol levels in rat blood at a level of 15%.

**Keywords:-** Effervescent Granules; Beetroot; Carrots; Apples; Cholesterol.

## I. INTRODUCTION

Hypercholesterolemia is a condition in which cholesterol concentration in the blood exceeds the standard value [1]. Cholesterol has been shown to disrupt and alter the structure of blood vessels resulting in impaired endothelial function leading to lesions, plaques, occlusions, and emboli. In addition, cholesterol is thought to be responsible for increased oxidative stress. Cholesterol in the food we eat can raise cholesterol levels in the blood, which results in hypercholesterolemia [2]. One of the most common diseases caused by increased cholesterol levels in the blood is atherosclerosis [1].

Some plants that have been used empirically by the community to reduce blood cholesterol levels are beetroot, apple and carrot, which can be used singly or in mixed form. The mixture of these three fruits is not only beneficial for lowering blood cholesterol levels but has also been used for hepatic protection and detoxification in cancer patients. In a single state, it can be eaten directly or made into juice, while in hybrid form, it is used by making the juice by cutting it into pieces and then mashing it through a coarse blender and filtering—the filter results in water that can be drunk once a day.

Apple extract (*Malus sylvestris* Mill) has been shown to reduce blood cholesterol in mice and potentially protect against cardiovascular disease [3]. Research on beetroot extract (*Beta vulgaris* L.) has been carried out [4], proving it can significantly reduce blood cholesterol levels in rats and at the same time have potential antioxidant activity. A study on carrot extract (*Daucus carota* L.) conducted [5] showed that carrots could reduce fat blood levels, including cholesterol and triglycerides, in albino rats.

According to [6], mixed juice of beetroot, carrots and apples can improve the nutritional quality of the three. Beetroot is very rich in antioxidants and natural nitrates. This nitrate will enhance blood flow throughout the body, including the brain, heart and muscles. This compound will increase nitric oxide molecules that help open blood vessels, increasing oxygen flow. These compounds also lower blood pressure, thereby reducing the risk of cardiovascular disease. But beetroot cannot be consumed too much, so it must be mixed with other natural ingredients such as carrots and apples. This study showed that the most stable mixture of beetroot, carrot and apple juice for 21 days was a mixture of 50:20:30.

To optimize the use of a mixture of these three plant ingredients, it is necessary to develop a pharmaceutical preparation that is stable, practical and attractive in use. One of them is granule preparation effervescent. This is to avoid damage from the water extracted by the air, so it does not have to be made new every day. As well as to increase the selling value of the stock.

This study is to analyze the potential of granule effervescent from a mixture of beetroot (*Beta vulgaris* L.), Malang apple (*Malus sylvestris* L.) and carrot (*Daucus carota* L.) extract using two sources of acid, namely citric acid and tartaric acid and a primary source of sodium bicarbonate in a ratio of 1: 1: 2. as preparation for lowering blood cholesterol levels as well as its evaluation in hypercholesterolemia male white rats.

## II. MATERIALS AND METHODS

This research is an experimental study with randomized and post-test control group designs [7].

The tools used are analytical scales, standard laboratory glassware, mortar and stamper, mouth mirror, pH meter, stopwatch. The materials needed are Beets, Malang apples and fresh carrots, Citric Acid, Tartaric Acid, Sodium bicarbonate, PVP, dextrin, aspartame and ginger extract, simvastatin, aqua, egg yolks, Wistar male white rats.

Each of the beetroot, Malang apple and carrot were extracted separately. The material is washed and then blended with enough water and filtered. The filter results are concentrated with a rotary evaporator. Then each extract was dried with 1:1 dextrin to obtain a dry extract of beetroot, Malang apple and carrot.

Preparation of effervescent granule formula used in this study is a modification from the previous study [8] by varying the levels of extract and levels of acid and base. The base mass is made by mixing the dry extract with sodium bicarbonate, powdered ginger flavoring and aspartame until homogeneous. PVP binder dissolved in 96% ethanol is added. After the mass can be clenched, it is then granulated. Acid mass is made by mixing powdered citric acid with tartaric acid until homogeneous. Add lactose and PVP binder in 96% ethanol grind until the mass can be clenched, then granulated.

Each granule of acid mass and alkaline mass was dried in a drying cabinet 1 x 24 hours. During the drying process, the granules are inverted.

After drying, it is sieved with 14 mesh then immediately dried again at a temperature of 40°C. The base mass and the mass were then mixed to evaluate the flow rate, angle of repose, dissolution time, high foam and ph.

TABLE 1. EFFERVESCENT GRANULE FORMULA COMBINATION

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Beetroot, Apple and Carrot	15%	20%	25%	-	15%	20%	25%	-
Citric Acid	500	500	500	500	500	500	500	500
Tartaric Acid	500	500	500	500	1000	1000	1000	1000
Na,Bicarbonate	1000	1000	1000	1000	1500	1500	1500	1500
PVP	800	800	800	800	800	800	800	800
Aspartame	60	60	60	60	60	60	60	60
Dextrin	600	800	1000	800	600	800	1000	800
Ginger Flavor	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Lactose	4000	4000	4000	4000	4000	4000	4000	4000

Effervescent granules from each formula were tested against hypercholesterolemia in male Wistar rats with simvastatin as a positive control. The number of rats is 36. The dose used for effervescent granules was 3.6 ml/200gBW, and the simvastatin dose was 0.18 mg/200gBW. The treatment was given on the 8th to the 14th day in rats fed egg yolk diet at a dose of 1.25 g/200gBW for 14 days. 4 grams of effervescent granules from each formula is dissolved in 200 ml of distilled water, then is left until the foam disappears. Simvastatin

suspension was prepared by dispersing 1 tablet of 500 mg simvastatin in 1% Na CMC solution.

After weighing the rats' body weight, the experimental animals were randomly divided into 9 groups, namely 6 treatment groups, 2 negative control groups and 1 positive control group. Each group consists of 4 rats. All animals in each group were given egg yolk for 14 days. Treatment on days 8-14 is as follows:

Group I : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution with 15% (F1) extract content.

Group II : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution with 20% (F2) extract content.

Group III: 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution with 25% (F3) extract content.

Group IV : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution with 15% (F5) extract content.

Group V : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution with 20% (F6) extract content.

Group VI : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution with an extract content of 25% (F7).

Group VII : 4 rats given egg yolk 1.25 g/200gBW and simvastatin 0.18 mg/200gBW (positive control).

Group VIII : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution without extract (F4) (negative control).

Group IX : 4 rats were given 1.25 g/200gBW egg yolk and 3.6 ml/200gBW effervescent granule solution without extract (F8) (negative control).

After the experimental animals were acclimatized for 2 weeks, they were given the following treatment:

- Rats are fed and drank as usual.
- 1.25 g/200gBW egg yolk is given for 14 days, and on the 7th day, total cholesterol levels were checked.
- From day 8 to day 14, each animal was treated according to the group.
- On the 15th day, rat blood was taken to check total cholesterol levels.

The results obtained are presented in tabular form and analyzed using pharmaceutical theory and one-way ANOVA statistics ( $p < 0.05$ ).

### III. RESULTS AND DISCUSSION

The following section describes the result and discussion of the study.

#### A. Extract Yield

Yield results of beetroot, Malang apple and carrot extracts are presented in Table 2.

TABLE 2. YIELD RESULT

Ingredient	Weight (g)	Thick Extract Weight (g)	Yield
Beetroot	1800	154.08	8.56%
Malang Apple	1930	241.7	12.52%
Carrot	2050	80.44	3.92%

The yield obtained for beetroot extract was 8.56%, carrot extract 3.92, and apple extract 12.52%. According to [9], apples using 96% ethanol is 11.67%, which means it is smaller than the water extract obtained in this study. For carrot extract extracted with water, according to [10], who in their research made effervescent tablets from carrot extract, the yield was 7.11%. Meanwhile, the beetroot extract obtained by [11] in their study was 8.39%, not much different from that obtained in this study.

#### B. Preparation Evaluation

Average physical evaluation results of respondents' preparations and responsiveness are presented in Table 3. The granule flow rate of all formulas ranged from 7.63 g/s to 9.80 g/s. This result is in the range of 4-10 g/sec, which indicates the granules can flow well. This situation will illustrate that when granules are produced on a large scale, the granules can flow nicely into the packages that have been provided so that the weight per package will meet the requirements for uniformity of weight. And if the weight meets the requirements, the dose per package will also follow what the manufacturer wants. In the measurement of the angle of repose of the granules, the lowest results were 24.94°, and the highest was 35.64°.

TABLE 3. PREPARATION EVALUATION

Evaluation	F1	F2	F3	F4	F5	F6	F7	F8
Flow Rate (g/sec)	8.84	8.55	7.63	9.34	8.77	8.69	8.13	9.80
The angle of Rest (degrees)	28.50	30.14	35.64	26.07	26.57	26.78	30.08	24.94
Compressibility (%)	22.90	21.84	20.83	22.92	21.96	20.87	18.74	25.48
pH	4.42	5.26	5.59	4.33	4.88	5.43	5.74	4.76
Solubility in Water 10°C	2.24	2.57	3.16	1.80	3.15	3.32	3.75	1.73
Solubility in Water 27°C	1.50	1.64	1.76	1.18	1.77	1.82	2.11	1.61
Solubility in Water 60°C	0.43	1.40	1.37	0.26	1.09	1.19	1.22	0.48
% Foam	10.70	9.91	9.09	27.54	21.26	18.69	15.97	25.37
Clarity	100%	100%	100%	100%	100%	100%	100%	100%
Flavor	50%	73%	20%	53%	67%	63%	67%	67%

According to researchers [12], if the angle of repose of the granules or powder is  $< 25^\circ$ , then the granule or powder flows very well. If the angle of repose is  $25^\circ$ - $30^\circ$  it flows well and if it is  $30^\circ$ - $35^\circ$ , the granules or powder flows well. F8 with an angle of repose of  $24.94^\circ$  is classified as very good, F3 with an angle of repose of  $35.64^\circ$  is quite good, and the others are classified as good. Thus, it is ensured that almost all the granules meet the requirements of the angle of repose.

Compressibility is the ability of the granules to remain compact under pressure. The direction of this evaluation is actually if the granules are to be compressed into tablets. However, if these preparations are produced in granules, the aim is more to the pressure obtained during packaging and packing of the preparations in primary, secondary or tertiary containers. From the evaluation results, the granule compressibility of all formulas was found to range from 18.74% to 25.48%. According to standard [13], the compressibility of 16-20% is considered reasonable, and 21-25% is classified as reasonably appropriate. The F7 preparation is quite good from these results, and other formulas are relatively good incompressible.

The evaluation results of the pH of the granules dissolved in water in a ratio of 4 grams in 200 ml of water, the lowest pH was 4.33 at F4, and the highest pH was 5.74 at F7. When viewed from the pH stability of beetroot extract is 4-8, carrot and apple extracts are around 4-5, the pH of all preparations can support the strength of the extracted content in it. Because the effervescent granule formula itself already contains acidulate as a source of acid, it can be estimated that the granules that have been dissolved in water will have an acidic pH.

Testing the dissolution time was carried out by dissolving 4 grams of granules in 200 ml of water at a temperature of 10°C represents cold temperature, 27°C represents normal water and 60°C means warm water. Of these three glasses of water, all preparations dissolve the fastest in water at 60°C and the longest at 10°C. The results of these three evaluations are no more than 5 minutes as a condition for good effervescent granule dissolution time. Control formulas are, on average faster than formulas containing extracts. Among formulas containing extracts, F1 dissolves the fastest at all temperatures.

All control formulas had more foam than formulas containing extracts for high foam. This is because beetroot extract, carrot extract and apple extract are more acidic, reducing the foam produced by the acidulating and carbonate base reactions. Formulas with more minor extracts have more foam, such as F1 and F5.

For the assessment of taste, smell and clarity of the preparation carried out through respondents as many as 30 people. All respondents who agreed to sign the informed consent form and evaluate the preparation can be done. Respondents have been given an explanation of what they will do and the researcher's actions if things go wrong. All preparations smell good, and all preparations are declared clear. 73% of respondents stated that the F2 granules were delicious, with a mixture of 20% extracts.

### C. Preparation Effectiveness

After testing the physical stability of the preparation, it was continued with a test of the pharmacological effect of the effervescent granule preparation containing a 1:1:1 mixture, water extract of beetroot, carrot and apple with levels of 15%, 20% and 25% respectively for the content of citric acid, tartaric acid and sodium bicarbonate in a ratio of 1:1:1 and 1:2:3. The anticholesterol effect of the preparation was tested on 8 groups of white male rats of the Wistar strain, each group of 4, plus 1 group that was given a positive control of simvastatin.

In each formula group there is also a control formula without extract.

Simvastatin administration was able to lower cholesterol levels better than a mixture of extracts. All groups treated with effervescent granule preparations containing a mixture of beetroot, carrot and apple extracts at levels of 15%, 20% and 25% (groups 1 to 6) showed a significantly different reduction in cholesterol levels with the group given granules without extract (groups 1 to 6, 8 and 9) and also with the simvastatin-positive control group (group 7). But the increase in the levels of the extract mixture in the preparation did not provide a significant difference in reducing blood cholesterol levels in rats. So the recommended dose is the dose with the lowest level of 15%.

Consuming beetroot can lower blood pressure to reduce the risk of cardiovascular disease [6]. Beetroot is very rich in antioxidants and natural nitrates. This nitrate will improve blood flow throughout the body, including the brain, heart and muscles. This compound will increase nitric oxide molecules that help open blood vessels, increasing oxygen flow. These compounds also lower blood pressure, thereby reducing the risk of cardiovascular disease. But beetroot cannot be consumed too much because it can increase blood sugar levels and uric acid levels, harm the fetus and burden the liver. So it needs to be mixed with other natural ingredients such as carrots and apples.

A mixture of beetroot, carrot and apple water extracts has several advantages in lowering blood cholesterol compared to being used separately. Beetroot contains betacyanin compounds, antioxidants that are very useful in controlling the increase in LDL and improving the system immune. Carrots contain beta-carotene, which can reduce fat levels in the liver and improve the cardiovascular system immune. Apples are high in fiber which can obstruct absorption of fat and cholesterol in the gastrointestinal tract. They contain quercetin and high vitamin C, an antioxidant that can bind free radicals that harm the body and build the system immune. So that the use of a mixture of these three extracts is beneficial for lowering cholesterol and can build body defenses and detoxify the liver [14].



#### IV. CONCLUSIONS

The physical examination of the effervescent granules containing the aqueous extract of beetroot, Malang apple and carrot met the requirements of the physical test of the preparation. And the results of testing on mice, it was found that all effervescent granule formulas containing mixed extracts of beetroot, apple and carrot extract (1:1:1) with levels of 15%, 20% and 25% can reduce blood cholesterol levels in mice differently from negative controls, but there is no difference between formulas. There was a difference in the effect of all formulas with simvastatin as the positive control, but the result did not exceed. It can be concluded that granule effervescent from a mixture of aqueous extracts of beetroot (*Beta vulgaris* L), Malang apple (*Malus sylvestris* L.) and carrots (*Daucus carota* L.) (1:1:1) can be used as preparations for lowering blood cholesterol levels at a level of 15%.

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