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Potential Antibacterial Activity of Granul Effervescent 4th Fraction of Taro Leaf Ethanol (*Colocacia esculenta* L.) Against Gram Negative Bacteria

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ABSTRACT
Background: The use of medicinal plants as traditional medicine in Indonesia has increased from year to year. One of the plants that can be used as a medicinal plant is the leaves of taro (*Colocacia esculenta* L.). This study aims to find out the potential antibacterial activity of granule effervescent 4th fraction of taro leaf ethanol extract (*Colocacia esculenta L.)* against Gram Negative bacteria on Pharmaceutical, KLT-Bioautography, and Agar Diffusion.
Result: The results of the 4th fraction effervescent granule test pharmaceutical using wet granulation have good pharmaceutic properties based on the silent angle test = 32.087°, flow time = 7.687 seconds, alir speed = 4.772 g/s and foam stopping time = 32.33. The results of Effervescent granule antibacterial activity tests on Bioautography-TLC provided activity against *Salmobella thypi*, *Eschericia coli* and *vibrio cholerae* bacteria at Rf value 0.74 and agar diffusion to obtain the largest hambat zone diameter against *Salmonella thypi* bacteria by 20.4 mm.
Conclusion: The results of the study found that the effervescent granules of taro leaves (*Colocacia esculenta* L.) had antibacterial activity with an Rf value of 0.74 and the diameter of the largest resistance zone against *Salmonnella thypi* bacteria of 20.4 mm.
KEYWORDS: Talas (*Colocacia esculenta* L.) Leaves, 4th Fraction Effervescent Granule, Antibacterial

I. INTRODUCTION

The utilization of medicinal plants in Indonesia is now starting to grow rapidly, seirin the more and more medicinal plants are used as traditional medicine. Medicinal plants are caused by the presence of compounds as secondary metabolites that are effective as medicinal compounds. New medicinal compounds from natural materials further clarify the important role of plant secondary metabolites as a source of medicinal raw materials. These secondary metabolites, proven to work as anticancer, antibacterial and natural antioxidants such as alkaloid compounds, tannins, polyphenol groups and derivatives whose use as natural remedies are believed to have relatively small side effects compared to modern drugs. Therefore, secondary metabolite compounds in their utilization are needed activity testing and pre-clinical testing even clinically to find out the efficacy, dosage and safety of natural compounds as antibacterial drugs.

One of the plants that is less scientifically utilized today is taro leaves (*Colocacia esculen*ta L.) which are used as inflammatory drugs, festering skin, blood,ulcers, and burns with chemical contentsaponin, terpenes, tannins, flavonoids, flobatanin, antraquinone, cardiac glycosides, and alkaloids (1). The compound has the potential to be used as a natural antibacterial for the prevention of pathogenic bacterial infections but scientifically the utilization of taro leaves (*Colocacia esculenta* L.) as a natural antibacterial drug has not been realized. In addition to the chemical content, taro leaves also have many nutrients, namely calcium, phosphorus, iron, vitamin C, thiamine, riboflavin (3). This type of Colocacia sp is one of the plants that grow in tropical areas both Asia, America, India and Africa. (4).

Previous research that the results of extract formulations as granule effervecsent have good pharmaceutic properties (2). Screening, chemical component and antibacterial activity based on resistance zone diameter at ethanol extract concentrations from taro leaves (*Colocacia esculenta* L.) against salmonella thypi pathogenic bacteria is potentially used as a natural antibacterial (5). With the activity of the taro leaf extract, it was tested the formulation and potential of effervecsent granules from taro leaves (*Colocacia esculenta* L.) against Gram Negative bacteria.

II. MATERIAL AND METHODS

1. Research Site

The research was conducted at the Laboratory of Pharmacaseutical and Microbiology, Faculty of Pharmacy, Moeslim University of Indonesia based on evaluation of effervescent granules as antibacterials by testing against pathogenic bacteria.

2. Research Materials and Tools

The material used by taro leaves (*Colocacia esculenta* L.) breeds pure pathogenic bacteria *Salmonella thypi*, *Vibrio cholerae*, *Eschericia coli* (ATTC 2019), DMSO (Dimethyl sulfoxide) (E.Merck), NaCl physiological 0.9%, medium NA (Agar Nutrient). The tools used are analytical scales, rotavapor, centrifuge, autoclave, oven, laminar water flow (LAF), incubator, UV spectrophotometry, dilution bottle, petri dish, spiritus lamp, water handler, rough scales, porcelain cup.

3. Research Prosedure

Taro leaves (*Colocacia esculenta* L.) are sororated with the aim of removing impurities in the sample by washing using running water then dried in the oven at 1050C for 60 minutes. Dry samples are extracted and formulated

3.1 Formulation and evaluation of effervescent granules

4th fraction of taro leaf ethanol extract (*Colocacia esculenta* L.) The result of fractionation is formulated in the form of effervescent granule preparations by means of fractions of 4 taro leaves (*Colocacia esculenta* L.) every 5 grams of preparation (1 sachet) of taro leaf fraction weighed as much as 1.5 grams, with the addition of lactose (0.75 g), sodium bicarbonate, citric acid, tartrate acid and sucrose. The formulation results are carried out the production of effervescent granule preparations based on organoleptic testing parameters, granule water content, flow time, stationary angle, dispersed ability, real type weight, compressed type weight and porosity.

3.2 Garnul effervescent

Effervescent granules are made using the wet granulation method. Lactose and extract are triturated in mortar until homogeneous. Tartrate acid, sodium bicarbonate and citric acid are each in the gerus to produce fine particles, then put into a mixture of lactose and extract and mixed until homogeneous. A PVP solution of K30 2% (b/v) is inserted into the powder mixture little by little and in wet water using mesh 10. The wet granules are then dried in the oven at 60^0C for 24 hours. Granules that have dried are then in the ayak using mesh 10 and conducted an evaluation of the physical properties of granules. The same is done for Formula 2.

3.3 Effervescent Granul Evaluation 4th fraction of taro leaf ethanol extract (*Colocacia esculenta* L.) (6.7, 9, 10,8,11,12)

a. Moisture Content* and *Loss on Drying

Granules that have been made are weighted wet before drying the granules and re-weighed after the granules are dried. Calculations of moisture content and loss on drying are calculated using equations:

$$MC = \frac{\text{Wet granule weight} - \text{heavy dried granules}}{\text{Weight of dried granules}} \text{x100}$$

$$\text{Loss on drying} = \frac{\text{Wet granule weight} - \text{heavy dried granules}}{\text{Wet granule weight}} \text{x100}$$

***b.* Bulk Density**

A total of 50.0 g of effervescent granules are inserted into a 50 mL measuring glass without being compressed. The powder is then measured the volume of real density, Vo. Bulk density is calculated using the following formula: ρb = M / Vo

***c.* Compressed Density**

After measuring bulk density, the sample was then compressed 500 times followed by an additional compression of 750 times until the difference between the measurement was less than 2% and then the volume compressed Vf, measured. Compressed density is calculated in gr/mL using the following formula: ρtap = M / Vf. Where, ρtap = compressed density, M = sample weight, Vf = compressed powder volume

***d. Carr's Index* (%)**

The compression index (Carr's index) is a measurement of powder propensity for the pump. The compressibility index is determined by bulk density and compressed density. In free-flowing granule powder, the interaction is generally less significant, and bulk and compressed density will be closer to the value. For hard-to-flow powders, large interparticle interactions, and large differences between bulk and compressed density were later observed. This difference is reflected in the Carr's Index which is calculated using the following formula: Compressibility Index = [(ρtap - ρb) / ρtap] / ×100. Where, ρb = Bulk Density, ρtap = Compressed Density

Hausner's ratio is an indirect index of the ease with which a powder flows. *Hausner's ratio* is calculated using the formula: *Hausner's Ratio* = Compressed Density (ρt) / Bulk Density (ρb)

Where ρt is compressed density and ρb is bulk density. Hausner's lower ratio (1.25) indicates a better flow than a higher value, between 1.25 to 1.5 indicating a moderate flow and more than 1.5 indicating poor flow.

***e.* Silent Angle Test**

The fixed funnel method is used to measure the stationary angle. The funnel pairs at a certain height (h) on graph paper placed on a flat horizontal surface. The powder is carefully poured into the funnel that has been covered at the end of the funnel until it touches the end of the funnel. . The bottom of the covered funnel is opened to let the granules down onto the graph paper. The radius of the base of the cone mound is measured. The silent angle (θ) is calculated using the following formula: **Tan θ = h/r.** Where, θ = Silent Angle, h = Cone height, r = Radius of the base of the cone. A value of a stationary angle of ≤ 30° usually indicates a free-flowing material and an angle of ≥ 40° indicates a difficult-flowing material, a value of 25-30 indicates the best flow properties, 31-35 indicates good flow properties, 36-40 indicates a relatively flowing flow and 41-45 indicates the nature of a flowing flow.

***f.* Foam Termination Time**

A total of 100 ml of distilled water is inserted in a 100 mL beaker, one dose of effervescent granules (2 grams) is poured into the beaker, the stopping time of the foam and the time produced froth is observed..

3.4 Testing of Effervescent granule 4th Fraction Taro Leaves Activity Against Gram Negative Bacteria *(Eschericia coli, Salmonella thypi dan Vibrio cholerae)* With Bioautography TLC And Agar Diffusion Method

a. Testing of Effervescent granule Activity Against Gram Negative Bacteria With Bioautography TLC Method

Granul effervescent 4th fraction ethanol extract of taro leaves (*Colocacia esculenta* L.) dissolved in chloroform:methanol (1:1) then attached to a thin-layer chromatography plate (KLT) measuring 7x1 cm, then diluted in a chamber containing eluent (phase of motion) n-hexan:ethyl acetate (10:1). After being traced on the plate is left to dry, then put in a petri dish, by placing it on the surface of a solid medium that is a nutrient medium so that the inoculated pathogenic *bacteria Salmonella typhi, Eschericia coli and Vibrio* cholerae are then allowed to diffuse for 60 minutes and then removed. Incubation at 37 oC for 1 x 24 hours and observed inhibitory zones against pathogenic bacteria on the medium and measured the rf value of chemical components on the KLT plate (15).

b. Testing Antibacterial Activity With Agar Diffusion Method

Medium NA sterile as much as 10 ml poured into a petri dish and allowed to solidify. A further 5 ml of NA medium that has been added 20 µl (0.02 ml) suspension of *pathogenic bacteria Salmonella typhi, Eschericia coli and Vibrio cholerae* are homogenized then poured and allowed to solidify. After that, antimicrobial susceptibility test discs are dissolved in a sample of effervescent granule extract ethanol taro leaves *(Colocacia esculenta L.)* at a concentration of 0.1%, 1%, 10% and put in a petri dish that has contained a medium. Inc incredated at 37oC for 24 hours. It then observed and measured the diameter of the resistance zone (13.16).

III. RESULT AND DISCUSSION

1. Formulation and Evaluation of Granul Effervescent 4th Fraction of Taro Leaf Ethanol Extract (*Colocacia esculenta* L.)

The results of the effervescent granule formulation of the 4th fraction of taro leaf ethanol extract (Colocacia esculenta L.) with the composition of the active ingredient formula are the 4th fraction of lactose taro leaves, PVP K30 (Dispersion 2% w/v), citric acid, tartrate acid and sodium bicarbonate, as seen in the following formula table.

Formula Composition	Formula 1	Formula 2
The Active Ingredient is the 4th Fraction of Taro Leaves	0.1 %	1%
Laktose	50%	50%
PVP K30 (Dispersion 2% w/v)	2%	2%
Tartrate Acid	2 gr	2 gr

Citric Acid	2 gr	2 gr
Sodium Bicarbonate	2 gr	2 gr

Table 1. Composition of Granul Effervecsent Formula 4th Fraction of Taro Leaves

Granules are particles that have a size of about 4-10 mesh. Granules are generally made by mixing powdered ingredients simultaneously and the hazelnut is moisturized to form a paste-like mass. The mass formed is then scuped and then dried in the air or in the oven. The effervescent granule formulation using monohydric citric acid and tartaric acid with a ratio of 1:2 produces powders with good effervescent properties. The combination of citric acid and tartaric acid is done because the use of a single monohydric citric acid will produce a sticky mixture that is not easily regranulated. While the use of single tartaric acid can produce granules that are too fragile and easily broken.

Effervescent is the release of gas bubbles from a liquid, as a result of a chemical reaction. The most common reaction for pharmaaceutic purposes is the acid-base reaction between sodium bicarbonate and citric acid (11). Citric acid : Tartaric acid : Sodium bicarbonate is 1:2:3. The results of the formulation of the 4th fraction granule as in the following figure.

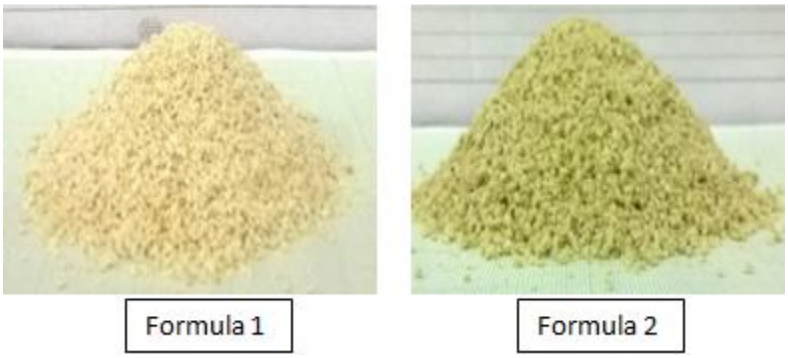

Figure 1. Results of Granulation of 4th Fraction of Taro Leaf Ethanol Extract

1. Granulation results from the 4th fraction of the base daunt ethanol extract (*Colocacia esculenta* L.)Hasil Uji Kelembaban (*Moisture content*) dan *Loss on Drying*

Formula	Heavy wet(g)	Dry Weight (g)	*Moisture Content* (%)	*Loss on Drying* (%)
1	00,017	4,517	8,339	5,497
2	8,441	6,721	13,515	1,906

Table 2. Bulk density test results effervescent granules 4th fraction of taro leaf ethanol (*Colocasia esculenta* L.)

2. Bulk Density Test Results

Formula	Weight (g)	Volume (mL)	Density (g/mL)
1	1,223	50,0	0,424
2	21,753	50,0	0,435

Table 3. 4th fraction effervescent fraction bulk density test results

3. Compressed Density Test Results

Formula	Heavy (g)	Volume (mL)	Compressed density (g/mL)
1	21,223	45,33	0,4682
2	21,753	46,33	0,469

Table 4. Results of compression density study of 4th fraction effervescent granules

4. True Density Test Results

Formula	Granule weight (g)	Granule volume (cm^3)	True density (g/cm^3)
1	13,133	12,663	1,0371
2	14,113	14,031	1,0058

Table 5. Results of true density study of 4th fractional effervescent granules

5. Compressibility Index Test Results (*Carr's Index*)

Formula	Densitas *bulk* (mL)	Compressed density (mL)	Compressibility index (%)
1	0,424	0,4682	9,440
2	0,435	0,469	7,249

Table 6. Results of research index compression of granul effervescent fraction 4^{th}

6. Test Results *Hausner's ratio*

Formula	Compressed density (mL)	Bulk density (mL)	*Hausner's Ratio*
1	0,468	0,424	1,104
2	0,469	0,4435	1,078

Table 7. The result of Hausner's ratio granul effervescent fraction 4^{th}

7. Silent Angle Test Results

Formula	Diameter (cm)	Tall (cm)	Silent Angle
1	10,93	7,134	33,144˚
2	11,03	6,934	32,087˚

Table 8. Results of silent angle test of 4th fraction effervescent granule

8. Flow Time And Flow Speed Test Results

Formula	Heavy (g)	Flow time (second)	Flow speed (g/second)
1	50,0	10,270	4,869
2	50,0	7,687	6,504

Tabel 9. Flow time test results and 4th fraction effervescent granule flow speed test

9. Foam Stop Time Test Results

Formula	Heavy (g)	Froth Stop Time (second)
1	2,0	96,00
2	2,0	32,22

Tabel 10. Results Test of 4th fraction effervescent granul Stop time Foam

The method of making effervescent granules uses the wet granulation method. This method of granulation has the advantage that fractional homogeneity is more homogeneous compared to other granulation methods. The resulting granule color is ivory white to dark green depending on the concentration of the extract fraction used. The higher the concentration of the extract fraction, the greener the granule color produced After obtaining the effervescent granules, the evaluation of granule physics to find out the physical properties and properties of granules.

Moisture content testing and loss on drying are important factors in solid preparations. The moisture content affects the granule flow time into the final container and the degrading speed of the granule. Moisture content is the total amount of water content in a preparation. From the results obtained the range of humidity levels from formulas 1 and 2 ranges from 8.339 % - 13.515 % (table 2), with the lowest humidity content in formula 1 which is 8.339%. Loss on drying is a parameter of water loss from the preparation as long as the granules are dried. Loss on drying determines the dryness of granules obtained. The greater the water loss from a stock, the drier and brittle the available obtained. From the results obtained resulted the range of loss on drying from 5.497% - 1.906%, with the largest loss on drying in formula 1 which is 1.906% (see table 2).

Density testing is defined as weight per unit volume. Bulk density ρb, defenisikan as the weight of the powder divided by the bulk volume and expressed as g/cm. Bulk density mainly depends on the distribution of particle size, the shape of particles and the tendency of particles to stick to each other. Bulk density is very important in terms related to the size of containers required for handling, shipping, storage of raw materials and mixing materials. Test results (table 3) show that formulas 1 and 2 have lower bulk densities ranging from 0.424 to 0.435 g/ml, and it can be assumed that granules obtained require a small container. While the compressed density (table 4) is similar to the bulk density result which ranges from 0.468 - 0.469 g / ml. The proximity of values between bulk density and compressed density indicates that interparticle interactions are less common. Proximity of bulk density values and compressed density will be seen there is a value of the compression index (*Carr's index*).

Another test result is that the compression index (Carr's index) is a measurement of powder propensity to be pumped. The compressibility index is determined by bulk density and compressed density. The smaller the ability can be pumped from a material the more it can flow the material. Test results found that the compressibility index values of all formulas ranged from 7.249% in formula 2 and 9.440% in formula 1 (table 6) included in the values with granule flow properties that had the best flow ability according to the requirements of 5-15% (14). Hausner Ratio testing is an indirect index of the ease of flow of powder. From the results obtained (table 7) you can see that all formulas have Haussner's ratio of less than 1.25 which indicates that the effervescent granules obtained have a good flow.

The silent angle test on formulas 1 and 2 is 33.144^o; 32,087^o (table 8). The value indicates that garnul has a good flow. The hausner ratio in all formulas shows less than 10. The value means that the granule has the best flow properties. Carr's Index of all formulas indicates that effervescent granules have excellent flow. Flow time testing and the speed of effervescent granule flow are affected by interparticle frictional forces. Flow time and flow speed are affected by the magnitude of the stationary angle, the compression index and Hausnerr's ratio. The smaller the stationary angle, the more the compressibility index value and Hausnerr's ratio of less than 1.25 then the flow speed is good. The speed of granule flow affects the number of granules that will flow into the container. The amount of granules that enter the container affects the number of doses to be consumed by the patient. From the results obtained (table 9) shows.

2. Testing of Antibacterial Activity of Granul Effervecsent 4th Fraction of Taro Leaves (*Colocacia esculenta* L.) *Salmonella thypi, Escheriacia Coli* and *Vibrio cholerae* by KLT-Bioautography

Granul effervecsent 4th fraction of ethanol extract taro (*Colocacia esculenta* L.) leaves obtained from granulation results namely formulas 1 and 2, conducted testing of antibacterial activity in KLT-Bioautography using eluent n-hexan: ethyl acetaata (10:1) obtained value Rf 0.74 provides activity against *Salmonella thypi, Escheriacia coli and Vibrio cholerae* bacteria, seen in table 11.

Rf	Spotting handler		Test Bacteria
	UV 254 nm	UV 366 nm	
0.74	yellow	Purpel	*Salmonella thypi*, *Eschericia coli* and *Vibrio cholerae*

Tabel 11. Testing of Antibacterial Activity of Granul Effervecsent 4th Fraction of Taro Leaves (Colocacia esculenta L.) Salmonella thypi, Escheriacia Coli and Vibrio cholerae by KLT-Bioautography

3. Testing antibacterial activity of Granul Effervecsent 4th Fraction of Taro Leaves (*Colocacia esculenta* L.) To *Salmonella thypi, Escheriacia Coli* and *Vibrio cholerae* On Agar Diffusion

Granule effervecsent 4th fraction ethanol extract taro (*Colocacia esculenta* L.) leaves obtained from granulation results namely formulas 1 and 2, tested antibacterial activity on agar diffusion in order to use a consensual variation of 0.1% and 1% provide activity against *Salmonella thypi, Escheriacia coli* and *Vibrio cholerae* bacteria obtained the largest bland zone diameter in formula 2 which is 20.4 mm against salmonella thypi pathogenic bacteria, as seen in table 12

Formula	Diameter of The Hambat Zone (mm)		
	Salmoella thypi	*Eschericia coli*	*Vibrio cholerae*
1	16	14	16
	17	13	17
	17	14	16
	16.7	13.7	16.4
2	20	18	19
	21	17	17
	20	18	19
	20.4	17.6	18.4

Table 12. Testing of Antibacterial Activity of Granul Effervecsent 4th Fraction of Taro Leaves (*Colocacia esculen*ta L.) To *Salmonella thypi*, *Escheriacia coli* and *Vibrio cholerae* On Agar diffusion

Testing of antibacterial activity of granul effervecsent 4th Fraction of taro leaves (*Colocacia esculenta* L.) to *Salmonella thypi*, *Escheriacia Coli* and *Vibrio cholerae* on agar diffusion. Based on the determination of the category of the diameter of the bland zone of an antimicrobial activity that the interpretation in the determination of the diameter of the antibacterial bland zone is categorized into three categories of activity by looking at the magnitude of the clear zone that is weak if the ≤ 11 mm, intermediate/medium if it ranges from 12-21 mm, and strong if ≥ 22 mm. The amount of the bland zone area formed is a clue to the sensitivity of microorganisms to the resulting antimicrobial compounds (17,18). The results of the activity test of effervescent granules 4th fraction of taro leaves obtained as 20.4 mm is the diameter of the intermediate/medium category bland zone. Antimicrobial ingredients with large clear zones are antimicrobial power ingredients or compounds are very good (19).

IV. CONCLUSION

Based on the results of the study it was concluded that :

1. Effervescent granules pharmaceutically using wet granulation have good pharmacopoictic properties based on the test of still angle, flow time, flow speed and foam termination time.
2. Granule effervescent Bioautography-TLC provides activity against the bacteria *Salmobella thypi*, *Eschericia coli* and *Vibrio cholerae* at a value of Rf 0.74 and on agar diffusion to obtain the largest diameter of the hambat zone against *Salmonelle thypi* bacteria of 20.4 mm.

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