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Optimization of Formula Granule of Lempuyang Gajah (*Zingiber zerumbet* (L) J.E.Smith) Rhizome Purified Extract as a Larvicide

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ABSTRACT

Lempuyang gajah rhizome (*Zingiber zerumbet* (L) Smith is considered potential as larvicidal. A previous study has shown that the purified extract of *Z. zerumbet* rhizome was toxic against *Adese aegypti* larvae. The aim of the study it to formulate a purified extract of *Z. zerumbet* (L) Smith in granule preparations by combining Sodium starch glycolate, PVP K-30 and tween 80. The granule formulation was optimized by the *Simplex Lattice Design* (SLD) method with using *Design expert* program 7.1.5. The results showed that the interaction of the three components can increase the flow rate index, the angle of repose, and reduce absorption. The optimum formula obtained was Sodium starch glycolate 2%, PVP K-30 2%, and Tween 80 5%. The analysis of one sample t-test shows that there is no significant difference between the predicted parameter values and the experimental results of the flow rate index and angle of repose, while the absorption response is significantly different. The optimum formula for granules has larvicidal activity with 100% larval death during 12-hour treatment.
Keywords: Lempuyang gajah; granul; larvicide

INTRODUCTION

An environmentally safe insecticide is selectively toxic to its target and is biodegradable, hence it could be utilized in target insect controlling program management. Natural bioactive product with larvicide potential should be developed as an alternative for larva control

Lempuyang gajah (*Zingiber zerumbet* (L) J.E.Smith) exists among the plants with a larvicidal activity against *Aedes aegypti.* Research conducted by Murini *et al*., (2017) shows that purified extracts of *Z. zerumbet* rhizome are soluble in petroleum ether and have larvicidal activity in *Ae.aegypti* mosquito larvae LC50 = 67.01 and LC90 = 90.0 ppm, and the extract has been standardized (Table I).

It is widely acknowledged that a formulation strongly influences the bioavailability of a product. Purified extracts that are soluble in petroleum ether are poorly dissolved in water, so additional ingredients are needed to facilitate water solubility. The purified extract of *Z.* According to Kala *et al.,* (2014), the liquisolid technique can treat poorly or very highly water-soluble pharmaceutical ingredients prepared for a solid dosage form (granules or tablets). In such a technique, the water-insoluble substance is dissolved in a non-volatile solvent to obtain a solution or suspension followed by the addition of an excipient to produce free-flowing, non-adherent, dry powder (Gangode, *et al.*, 2016). The formula of granules from the purified extract of *Z. zerumbet* rhizome consists of natrium starch glycolate as a disintegrant, polyvinylpyrrolidone K-30 (PVP K-30) as a binder, Tween 80 as a solubilizer, and lactose as a filler. Tween 80 as a non-volatile liquid vehicle in the liquisolid technique is used to enhance the solubility of active ingredients. This technique also involves PVP K-30 functioning not only as a binder but also as a solubility enhancer, film-forming agent, and dispersant (Chandel *et al.*, 2013). The component materials greatly affect the stability of granule dosage form, and the proportions of each should be optimized to obtain the desired properties of a granule. One of the methods for this optimization is the *Simplex Lattice Design* (SLD) which can optimize formulas with varied compositions of different component materials. This method is practical and quick as it does not apply trial and error formulations (Bolton, 1997).

This study aims to determine the proportion of a mixture of Na starch glycolat, PVP K-30 and tween 80 to produce purified Z.Z rhizome extract granules with expected physical properties.

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Table I. Kinds of parameter from the purified extract of Z. *zerumbet* rhizome

Kind of parameters standardization	Purified extract
Organoleptic	
colour	dark brown
smel	specific
taste	bitter
Total ash contain	3,46%
Water soluble compounds	4,46%
Ethanol soluble compounds	0,16%
Drying shrinkage	4,0%
Stickess	< 1 secon/80g
Viscosity	230±8,16 seconds
Active substance concentration	21,11±2,19%

METHODOLOGY

Materials

Lempuyang gajah (*Z. zerumbet*) rhizome was obtained from Jatimulyo, Kulon Progo District of Yogyakarta, in May 2014. The rhizome was authenticated at the Department of Pharmaceutical Biology (BF/284/Ident/Det/VI/2014), the Faculty of Pharmacy of Universitas Gadjah Mada, Yogyakarta – Indonesia. All the organic solvents were of pro-analytical grade, and the instar larvae III-IV of *Ae. aegypti* were obtained from the Parasitology Laboratory of the Faculty of Medicine, Public Health and Nursing, School of Medicine, Universitas Gadjah Mada according to standard. The materials for granule production were lactose (Meggle), sodium starch glycolate (Thianjin ILE), PVP K-30 (BASH SE), and tween 80 (Brataco,chemical), Abate (Bash Indonesia).

The instruments used were *MB35 Halogen Moisture Analyzer Ohaus, abrasive tester* (Erweka) Tipe T.A.P, Jerman, sentrifuge (Hitachi 18PR/5).

Methods

Preparations for purified extracts

Five hundred grams of *Z. zerumbet* rhizome dried powder extracted by maceration for 24 hours at room temperature using 1 liter of methanol. Filtering is done using a buchner funnel and the pulp is macerated again in the same way twice then filtered. The obtained filtrate was evaporated, and that thick methanol extract was obtained. The success of extraction was monitored by Thin Layer Chromatography (TLC) method. Twenty-five grams of methanol extract of *Z. zerumbet* rhizome was purified using 200 ml of petroleum ether (PE) and centrifuged at 5000 rpm for 15 minutes, to obtain PE supernatant containing compounds soluble PE and pellets containing insoluble PE compounds. The supernatant was evaporated to obtain a PE soluble purified extract. The success of this search was monitored by the thin layer chromatography (TLC) method. Furthermore, two components of both soluble and insoluble PE were tested for activity against *Ae aegypty* larvae.

Characterization of purified extract of *Z zerumbet* rhizoma

The characteristics purified extract of the *Z. zerumbet* which was determined included non-specific parameters and specific (organoleptic, adhesion, viscosity, and active substance content).

Formula of purified extract *Z.zerumbet* granule, every 200 mg of granule contains 20 mg of purified extract. Optimization formula is done using the *Simplex Lattice Design* method with software *Design expert* 7.1.5 programe (Tabel II)

Preparation of granule dosage form

Each ingredient was weighed, and PVP K-30 was dissolved with water as required (mixture 1) while the Natrium starch glycolate was mixed with lactose to obtain a homogenous solution (mixture 2). The purified extract of *Z. zerumbet* rhizome and Tween 80 were combined into a homogenous mixture, and mixture 2 was added followed by the addition of mixture 1 to produce granule mass. After passing an 18-mesh sieve, the granule mass was stored in a drying chamber at a temperature of 50^{o}C. The dry granule was then sieved with No. 25 mesh but not able to pass No. 30 mesh followed by testing the flow rate index, angle of repose, and absorption.

Test of the physical properties of granules purified extract of *Z. zerumbet* rhizome

Angle of repose

Flow properties of the physical mixtures of all the formulations were determined by calculating the angle of repose by fixed height method. A funnel with 10 mm inner diameter of the stem was fixed at a height of 2 cm. Over the

Table II. Granule composition from the purified extract of *Z. zerumbet* rhizome

Run	Purified extract of Zz rhizome (%)	A Na Starch glycolate (%)	B (PVP K-30) (%)	C (Tween 80) (%)	Lactose (%)
1	10	2,5	4	2,5	81
2	10	2	3,5	3,5	81
3	10	5	2	2	81
4	10	3	3	3	81
5	10	2	2	5	81
6	10	2	2	5	81
7	10	2	5	2	81
8	10	2	5	2	81
9	10	5	2	2	81
10	10	2,5	2,5	4	81
11	10	3,5	3,5	2	81
12	10	4	2,5	2,5	81
13	10	3,5	2	3,5	81

platform. About 50 grams of the sample was slowly passed along the wall of the funnel till the tip of the pile formed and touches the steam of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured

The flow rate

A glass funnel was fixed at a 10 cm height over a flat horizontal was temporarily blocked with a flat ruler. A clean white paper was spread beneath the funnel on the horizontal surface for collection of the powder. A 50-gram quantity of the sample was then weighed and poured into the funnel, the exit of the funnel was then sharply unblocked and the time (seconds) taken for the sample completely flow out onto the horizontal surface was measured with a stopped clock.

Absorption Test

Each of the granule formulas was weighed for 0.5 grams and placed on Hirsch-Enslin funnel, then the amount of water absorbed was recorded at 5-minute intervals by reading the scale on the equipment. The test was performed for 30 minutes followed by making a curve of the correlation between the amount of absorbed water and time (minute).

Larvicidal bioassay

Preparation of *Ae. aegypti* colony

Colonization of *Ae. aegypti* was done in the Parasitology Laboratory, Faculty of Medicine, Public Health, and Nursing UGM. Adult *Ae. aegypti* was put in a cage completed with an aspirator and a cup containing water for the mosquito to lay eggs. The *Ae. aegypti* eggs were then placed on a tray containing 1500 mL of water, and after 1-2 days the eggs hatched into larvae. During the process of colony formation, a chicken liver was added as the food. Identification of instar larvae III-IV of *Ae. aegypti* was performed 7 days after the eggs hatched, observed macroscopically and using a loupe whenever necessary.

Larvicidal Activity granules purified extract of *Z. zerumbet* rhizome against *Ae. aegypti*

Thirteen 200-ml containers were each filled with 100 ml of water, 25 larvae, and 200 mg of granule purified extract with various formulas. The observation was conducted threefold at 3-hour intervals to count the number of dead larvae per container. A larva is considered as dead if it sinks or shows no movements after being interrupted or touched with a pipette on the siphon or thorax area (WHO, 2005)

Analysis of results:

Data from the parameters of flow rate index, angle of repose, and absorption test were analyzed using the *Design Expert* 7.1.5 software. The determination of optimum formula was based on a *Superimposed Contour* plot of the parameters.

RESULT AND DISCUSSION

Purified methanol extracts with petroleum ether (PE): ethanol: water (28: 15: 5), were monitored by TLC with a mobile phase of ethyl acetate: wash benzene (1; 3) ((Figure 1).

Standardization must be performed to increase the quality of natural products. The standardization purified extract of the *Z. zerumbet* which was determined included non-specific and

Table III. Result of flow rate index, angle of repose, and absorption test on the granules of the purified extract of *Z. zerumbet* rhizome

The physical properties of granules of the purified extract of *Z. zerumbet*			
Run	**Flow rate index (g/second)**	**Angle of repose (°)**	**Absorption (mg/minute)**
1	8.3 ± 0.12	36 ± 0.82	19.45 ± 0.46
2	8.6 ± 0.36	33 ± 0.82	20.34 ± 0.71
3	7.8 ± 0.33	33 ± 0.82	18.65 ± 0.46
4	6.8 ± 0.09	39 ± 0.82	18.81 ± 1.10
5	10.7 ± 0.57	26 ± 0.82	16.52 ± 0.75
6	9.5 ± 0.54	26 ± 0.00	20.87 ± 0.65
7	9.2 ± 0.29	28 ± 0.82	29.79 ± 0.39
8	9.1 ± 0.16	28 ± 1.63	28.72 ± 0.49
9	8.8 ± 0.61	29 ± 0.82	21.26 ± 1.75
10	9.2 ± 0.26	28 ± 0.82	17.19 ± 0.78
11	6.3 ± 0.26	39 ± 0.82	14.94 ± 3.04
12	7.3 ± 0.43	33 ± 1.64	21.97 ± 1.18
13	8.1 ± 0.17	31 ± 1.41	24.97 ± 0.42
Model	Special cubic	Special cubic	Special cubic
Significant model	0.024 Significant	0.0269 Significant	0.0171 Significant
Lac of fit	0.5222 Insignificant	0.1694 Insignificant	0.4255 Insignificant

Figure 1. Chromatograms produced by preparative TLC with mobile phase ethyl acetate:WB (1: 3), stationary silica gel GF_{254} observed with UV light 254 A : Purified extract B : Metanol extract of *Z. zerumbet*

specific (organoleptic, stickiness, viscosity, and active substance content) (Table I).

Testing the physical properties of the granules based on the *Simplex Lattice Design* (SLD) from the *Design Expert* software analysis is presented in Table III. Based on the data obtained, can be made contour plots and mathematical equations. The results obtained can be used to predict the value of the response theoretically. Equations related, Y = a (A) + b (B) + c (C) -ab(A)(B) + ac(A)(C) + bc(B)(C) + abc (A) (B) (C)

Y = measured response; a,b,c,ab,bc,abc = coefficient; A = sodium starch glycolate; B =PVP K-30 and C= tween 80.

a. Flow rate index

According to the flow rate index, the following equation would be obtained: (equation 1)

Y = 3.17(A) + 3.31(B) + 2.45(C) - 1.23(A)(B) - 0.64(A)(C) - 0.55(B)(C) + 0.09(A)(B)(C)

The equation above indicates that each component and its interactions influenced the flow rate of granule mass. PVP K-30 was proved the most dominant component affecting the flow rate with a coefficient value of 3.31. Flow rate index granules purified extract of *Z. zerumbet* rhizome were also influenced by interactions of two components. The interaction between Na. starch glycolate and PVP K-30 gave the most negative influence on the flow rate with a coefficient of 1.23 as opposed to the other two interactions. The interaction among the three components had brought a positive reaction towards the flow rate though with a small coefficient.

The flow rate index of the granules purified extract of *Z. zerumbet* rhizome showed that the interaction among Na starch glycolate, PVP K-30, and Tween 80 improved the granules. The factors affecting a particle size include particle shape, particle density, powder porosity, electrostatics, relative humidity, particle surface condition, chemical structure, and measurement method

(Voigt, 2005). A good granule mass has a flow rate index of > 10g/second (Banker *et al.,* 1986), while this study found a 10.42 g/second flow rate index. Although fulfilling only the minimum desired criteria, this result remained necessary for determining the dosage form to be prepared. Although tablet dosage form is not produced, the flow rate index would normally influence the non-uniform granule mass during the process of pouring granules into a sachet.

b. Angle of repose

The angle of repose describes the flow property of powder during a tableting process. The magnitude of the angle of repose depends on cohesion and friction between particles; the smaller the cohesion and friction, the granule will be faster and easier to flow. The flatter the pile, the lower the angle and the better the powder flow (Banker and Anderson, 1986).

The equation for the angle of repose is as follows:

$Y = -0.76(A) - 5.20(B) + 0.88(C) + 3.07(A)(B) - 0.39(A)(C) + 1.38(B)(C) + 0.55(A)(B)(C)$

This equation shows that each component and their interactions influenced the angle of repose of granules purified extract of *Z. zerumbet* rhizome. Natrium starch glycolate and PVP K-30 responded negatively to the angle of repose, while Tween-80 had a positive response by increasing the angle of repose. Interaction among Na starch glycolate – PVP K-30 and PVP K-30 – Tween 80 gave a positive response to the angle of repose, but Na starch glycolate – Tween 80 responded negatively by decreasing the angle of repose. Meanwhile, interaction among the three components could raise the angle of repose although the coefficient was small. The lower the angle of repose, the easier it is for the granule to flow freely. The angle of repose for optimum granule formula in this study reached 25.38^o. The angle of repose is closely related to the flow rate property; a good flow rate index will generally have a good angle of repose of < 40^o (Voigt, 2005). According to Patel and Shah (2016), the angle of repose of granules resulted from the liquisolid technique is better than those from a conventional formula due to the use of unevaporated solubilizer.

c. Absorption

The equation for absorption is:

$Y = 1.35(A) + 13.27(B) - 3.55(C) - 2.83(A)(B) + 3.77(A)(C) - 0.74(B)(C) - 0.61(A)(B)(C)$

This shows that each component and its interactions affected the absorption ability of the granules. Absorption with PVP K-30 increased better than with Na starch glycolate, reaching 13.27 coefficient value, but Tween 80 reduced the absorption. The absorption ability was also influenced by the interaction between two components. Natrium starch glycolate and PVP K-30 interaction caused more reduced absorption than the interaction between PVP K-30 and Tween 80, while Na starch glycolate and Tween 80 positively influenced the absorption with a coefficient 37.7. However, the interaction between the three components decreased the absorption with a coefficient of 0.61. The absorption test for the granules of the purified extract of *Z. zerumbet* rhizome showed a significantly different result from the predictions made using either *Superimposed Contour* plot or manual calculation. Absorption is an important property in the solubility of the active ingredient of preparation with water as the material of medium capable of diffusing active ingredients out of the system. Water absorption will affect the disintegration time of granules; the denser the particle arrangement, the longer the time taken by water to penetrate granules. This study found a relatively high absorption, which would lead to more rapid penetration of water into granules.

Determination of Optimum Formula

The optimum granule formula can be determined based on the SLD mathematical equations from the experiment with Y1 = flow rate index, Y2 = angle of repose, and Y3 = absorption. The *Superimposed Contour* plot was based on the contour plot of the obtained parameters to determine the optimum area and point.

Analysis from *Design Expert* software with SLD resulted in optimum formula (Figure 2) with 2%, 2%, and 5% concentrations of Na starch glycolate, PVP K-30, and Tween 80, respectively. The desirability of this recommended optimum formula reached 0.874. Desirability determines the expected result and ranges from 0 to 1. The value closer to 1 means that the recommended formula has met the desired criteria.

The optimum formula resulted from the *Superimposed Contour* plot was then put in SLD equation and calculated manually to obtain the predicted response value of an optimum formula.

The equations of the three parameters with manual calculation are as follows:

Flow rate index

$Y = 3.17(A) + 3.31(B) + 2.45(C) - 1.23(A)(B) - 0.64(A)(C) - 0.55(B)(C) + 0.09(A)(B)(C)$

$= 10.19$ g/second

Angle of repose

$Y = -0.76(A) - 5.20(B) + 0.88(C) + 3.07(A)(B) - 0.39(A)(C) + 1.38(B)(C) + 0.55(A)(B)(C)$

$= 25.8^o$

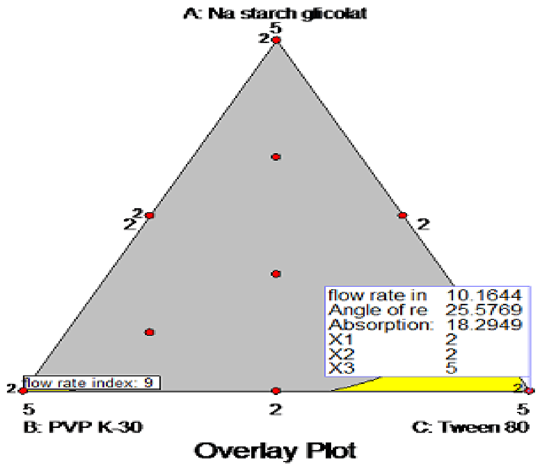

Figure 2. Superimposed from the contour plot response flow rate, angle of repose, and absorption granule of purified rhizome extract *Z.zerumbet* rhizome

Table IV. Verification of test result against manually calculated prediction

Response	Manual Calculation	Test Result	Significance	Conclusion
Flow rate index (g/second)	10.19	10.42± 0.38	0.492	Insignificant
Angle of repose (°)	25.66	25.38 ±0.33	0.606	Insignificant
Absorption (mg/minute)	18.27	27.90 ±0.63	0.004	Significant

Table V. Verification of test result against *Superimposed Contour* plot prediction

Response	*Superimposed Contour* Plot	Test Result	Significance	Conclusion
Flow rate index (g/second)	10.16	10.42 ± 0.38	0.555	Insignificant
Angle of repose (°)	25.60	25.38 ± 0.33	0.478	Insignificant
Absorption (mg/minute)	18.29	27.9 ± 0.63	0.004	Significant

Absorption

$$Y = 1.35(A) + 13.27(B) - 3.55(C) - 2.83(A)(B) + 3.77(A)(C) - 0.74(B)(C) - 0.61(A)(B)(C) = 27.9 \text{ mg/minute}$$

Verification

The verified formula consisted of Na starch glycolate (2%), PVP K-30 (2%), and Tween 80 (5%). Three types of responses resulted from the experiment of optimum granule formula were compared with the manually calculated prediction and presented in Table IV. Meanwhile, Table V describes a comparison between the response from the experiment of optimum granule formula and a prediction from the *Superimposed Contour* plot. The flow rate index and angle of repose belonging to the optimum formula of granules from the experiment were compared to those of the manually calculated prediction as well as *Superimposed Contour* plot prediction using T-test, resulting in an insignificant difference ($p>0.005$) while the absorption was otherwise ($p<0.05$).

Mortality rate (%) of *Ae. aegypti* after administration granules of purified extract of *Z. zerumbet* rhizome

Thirteen run granule purified extract of *Z. zerumbet* rhizome was tested against *Ae. aegypti* larvae and the percentage of larva mortality at a certain time is presented in Table VI.

Almost all of the run granules caused 100% mortality of *Ae. aegypti* larvae in 12 hours, except run 1, 7, 8, and 12. Run 12 granule led to 100% in 18 hours, while running 7 and 8 did in 24 hours and run 1 in 27 hours.

The optimum formula with three responses indicated that the interactions among the three components would improve the flow rate index and angle of repose but reduce absorption. Although absorption was low, the formula could kill all the larvae in 12 hours similarly to the positive control (Abate). Abate able to kill the larvae 100% on the hours to 12, and it can be shown that Abate had good larvicidal power. The active ingredient in the granules of the purified

Table VI. Percentage of larva mortality at various observation time

RUN	Larva *Ae.aegypti* mortality (%)								
	3 hours	**6 hours**	**9 hours**	**12 hours**	**15 hours**	**18 hours**	**21 hours**	**24 hours**	**27 hours**
1	6,7±1,89	16,0±3,27	56,0 ±3,27	60,0±3,27	68,0±3,27	77,3±1,88	89,3±1,89	96,0±3,27	100
2	13,3±1,89	22,7±1,89	40,0 ±3,27	100	-	-	-	-	-
3	6,8±1,89	20,0±3,27	85,3 ±1,89	100	-	-	-	-	-
4	0	10,7±3,77	72,0 ±3,27	100	-	-	-	-	-
5	18,7±1,89	24,0±3,27	72,0±3,27	100	-	-	-	-	-
6	17,3±1,89	34,7±1,89	54,7±1,88	100	-	-	-	-	-
7	5,3 ±1,89	8,0 ±3,27	26,7± 3,78	49,3±3,77	65,3±1,89	74,7±1,89	85,3± 1,89	100	-
8	0	2,7±1,89	33,3±1,89	50,7±3,77	69,3±3,77	77,3±4,99	81,3±1,89	100	-
9	5,3±1,89	26,7±2,3	80,0±3,27	100	-	-	-	-	-
10	0	12,0±3,27	73,3±1,89	100	-	-	-	-	-
11	5,3±1,89	13,3±1,89	52,0±3,27	100	-	-	-	-	-
12	0	21,3±3,77	56,0±3,27	69,3±1,89	89,3±1,89	100	-	-	-
13	6,7±1,89	16,0±3,27	60,0±3,27	100	-	-	-	-	-
Abate	9,3±1,89	20,0±3,27	70,7±1,88	100	-	-	-	-	-
Opti-mum	6,7±1,89	28,0±3,27	77,3±1,89	100	-	-	-	-	-

Note: Run 1-13: contains purified extract *Z. Zerumbet* rhizome and Na Starch glycolat, PVP K-30 and Tween 80 with various concentration; Optimum: granule optimum formula Na Starch glycolat (2%), PVP K-30 (2%) and Tween 8(5%)

extract of *Z. zerumbet* rhizome was a viscous extract solved in petroleum ether (PE) that could not mix with water. In this liquisolid technique, Tween 80 became a non-volatile liquid vehicle that could increase the solubility of active ingredient because the surface tension between the dissolution medium and granule surface was reduced. The lactose in granule formula functioned as a carrier, an ingredient with high porosity and absorption properties capable of absorbing the liquid of purified extract of *Z. zerumbet* rhizome dispersed in Tween 80. Meanwhile, PVP K-30 played the role not only as a binder but also as a solubility enhancer as well as a coating agent and dispersant. In the granule purified extract of *Z. zerumbet* rhizome, PVP K-30 would inhibit the crystallization caused by drug retention inside the porous cavity of an excipient during contact between granule dosage form and dissolution medium (Khan *et al.*, 2017). In contrast, Na starch glycolate functioned as a disintegrant to counterbalance PVP K-30.

Therefore, the granule optimum formula of the purified extract of *Z. zerumbet* rhizome consisting of Na Starch glycolate, PVP K-30, and Tween 80 at 2%, 2%, 5% concentration, respectively, was the best formula to be able to release the active ingredient.

CONCLUSION

The optimum formula of granule purified extract of *Z. zerumbet* rhizome was obtained in the proportion of Na starch glycolate 2%; PVP K-30 5% and Tween-80 5%. The optimum formula obtained has a flow rate index and angle of repose showing the same results as the predicted responses given by Design-Expert software version 7.1.5, while the absorption response shows significant results. The interactions among these three components could improve the flow rate index, increase the angle of repose, and decrease absorption.

The optimum formula granule of purified extract of Z. *zerumbet* obtained gave reaches to death 100% at 12 hours

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