

Antihypertensive Activities Instant Granul of Combination Extract Roselle Flower Petals (*Hibiscus sabdariffa* L.), Chayote Fruit (*Sechium edule* (Jacq.) Sw.) and Aloe Vera Leaves (*Aloe Vera* L.) in White Male Rats

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Abstract

Hypertension is a condition where the systole blood pressure is greater than 140 mmHg and diastole blood pressure is greater than 90 mmHg. The previous study showed that the combination of roselle flower petal *Hibiscus sabdariffa* L.), chayote fruit (*Sechium edule* (Jacq.) Sw.), aloe vera leaf (*Aloe vera* L.) extracts can reduced high blood pressure better than its single herb extract. The purpose of this research is to determine the optimal dose of instant granules from a combination of extracts of roselle flower petals (*Hibiscus sabdariffa* L.), chayote fruit (*Sechium edule* (Jacq.) Sw.), aloe vera leaf (*Aloe vera* L.) on white male rats *Rattus norvegicus* Sprague Dawley induced with adrenaline 1.2 mg/kg BW intraperitoneally using Non-Invasive Blood Pressure method.

The instant granule suspension preparation is administered orally at doses 0.5 g/200 g BW, 0.75 g/200 g BW and 1 g/200 g BW. The results showed that at the significance level of 0.05, all the instant granule doses tested have an activity of reduction of systolic and diastolic blood pressure respectively 30.27% and 31.86%, 21.77% and 21.27%, 18.39% and 23.05%.

Keywords: Hypertension, *Hibiscus sabdariffa* L., *Sechium edule* (Jacq.) Sw., *Aloe vera* L., Non-Invasive Blood Pressure.

Introduction

Hypertension is the most common cardiovascular disease. The prevalence of this disease increases with age. Increased arterial pressure causes pathological changes in vascular tissue and left ventricular hypertrophy⁴. Hypertension is a condition in which a systolic blood pressure greater than 140 mmHg and is accompanied by a rise in diastolic blood pressure greater than 90¹⁰.

According to WHO in the 2013¹⁷, hypertension is a global problem with a high number of patients. In the worldwide,

about 40% of adults aged 25 years or over have been diagnosed with hypertension. The number of hypertensive patients increased from 600 million in 1980 and approached one billion in 2008. According to the Basic Health Research (RISKESDAS) 2007², the prevalence of hypertension in Indonesian population with an age range of 18 to 29 years was 29.8%, the regions with the highest hypertension prevalence incident were Natuna (53.3%), Mamasa (53.6%) and Wonogiri (49.5%).

The usage of common antihypertensive drugs such as thiazide diuretics, adrenergic receptor blockers, calcium channel inhibitors and angiotensin-converting enzyme inhibitors are always followed by adverse side effects. These side effects include vertigo, depression, congestive heart failure, gastrointestinal disorders and even an increased risk of diabetes⁵. Therefore the public is more interested in using medicines derived from natural ingredients with lower side effects because people suffering from hypertension will use the drug for the rest of their lives¹⁴.

Plants as medicinal ingredients have been exploited by the Indonesian society since the ancient time. One of the herb widely used as the ingredients of medicine today is roselle flowers. In traditional medicine, roselle petals extract is used to treat several diseases including high blood pressure, liver disease and fever. In rats and rabbits, roselle petals extract showed antihypercholesterolemia, antinociceptive and antipyretic and showed antihypertensive activity¹. Based on research conducted by Faraji and Tarkhani,³ patients were given intravenous *Hibiscus sabdariffa* L. After 12 days, systolic blood pressure decreased by 11.2% and diastolic by 10.8% compared to blood pressure before administration. The results also showed that high anthocyanin content in roselle petals extract (*Hibiscus sabdariffa* L.) significantly reduced blood pressure and reduced plasma ACE (Angiotensin Converting Enzym) in hypertensive patients⁶.

In addition, the chayote squashes (*Sechium edule* (Jacq) Sw.) are an ingredient of vegetable empirically used to lower blood pressure. The community has already recognized the chayotes not only as a food ingredient that is processed into various variations of cuisine, but also as a

traditional medicine to lower blood pressure⁷ such as research conducted by Gordon et al⁵ that water-soluble soluble chayote extracts can lower blood pressure.

In a study conducted by Rinayanti et al,¹³ the provision of aloe vera juice in white rats (*Rattus norvegicus* L.) can reduce the frequency of heart rate. The decrease of heartbeat frequency will cause a decrease in blood pressure.

In a study conducted by Puspa et al,¹¹ it was found that the combination of roselle flower petals extract (240 mg/kg BW), chayote squash (250 mg/kg BW) and aloe vera leaf (100 mg/kg BW) can lower male white rat blood pressure of systolic blood of 18.31% and diastolic by 23.19% is better than single administration, Rahmawati et al¹² have obtained instant granule formula from the combination of roselle flower petals extract, chayote squash and aloe vera leaves that have good quality in accordance with the requirements of granules.

With this background, it is necessary to do further research to determine the optimal dosage of instant granules combination extract roselle flower petals, chayote squash and aloe vera leaves.

Material and Methods

Tools: The tools used in this study are commonly used equipment in the pharmacology laboratory such as blenders, macerators, glassware, rotary evaporator, analytical scale (Dragon 204), coarse scales (OHAUS), mesh 16 and 14 mesh, syringe 1 ml, 5ml oral sonde, animal scales and digital tension meter (CODA™ Non Invasive Blood Pressure Kent Scientific Corporation) and other tools commonly used in Pharmaceutical Laboratory of Natural Materials and Pharmacology Faculty of Pharmacy Universitas Padjadjaran.

Materials: The materials used in this study include simplicia roselle flower petals (*Hibiscus sabdariffa* L.), fresh chayote squash (*Sechium edule* (Jacq. Sw.)), fresh aloe vera leaves (*Aloe vera* L.) from "Perkebunan Tanaman rempah dan Obat Manoko, Lembang Bandung, ethanol 70% (Bratachem), maltodextrin (Bratachem), sucrose, acesulfame (quadrant), aquadest, adrenaline, captopril, gom arab are material from pharmacology laboratory and 20 male white rats Sprague dawley.

Methods

Extraction: The process of simplicia extraction of roselle petals is done by maceration method using ethanol 70%. Furthermore, the water extract obtained by maceration method is thickened with a rotary evaporator at a temperature of 40°C. The fruits of dried squash and dried tongue leaves are dried using freeze drying previously crushed using a blender separately.

Making of Instant Granules: The instant granule formula obtained from Rahmawati et al¹² can be seen in table 1.

The steps to make instant granule were: dried Aloe gel, maltodextrin, sucrose, acesulfame and benzoic acid in a sieve, then weighed. Extracts of roselle flower petals and chayote squash are dried with maltodextrin. After that, mix with sucrose, acesulfame and benzoic acid in one container. The mixture was sieved through a mesh screen,¹² then the obtained wet granules were dried in an oven at 40-45°C for 18 hours. The granulated drying results are sieved back with mesh⁹. Granules are inserted into desiccators containing silica gel to avoid moisture absorption from the air before packing in aluminum foil containers.

Antihypertensive Activity Testing

1. Animal Test Preparation: Prior to the testing, the experimental rats have to adapt first in the test room for approximately 7 days. Furthermore, allow rats to adapt in the animal holder at least 15 minutes for 3 consecutive days. During adaptation, every day rats are weighed and checked for health. The characteristics of the rats are healthy, physical appearance is clean, active and weight does not fall more than 10%.

2. Preparation of Dosage Forms Test: The tested preparations that had been used in this experiment were PGA suspension with 2% dose of 4.5 mg/kg BW and suspension of instant granule made by the addition of PGA as much as 2% with three dosages 0.5 g/200 g BB, 0.75 g/200 g BB and 1 g/200 g BB.

3. Rats Classification: Rats were grouped using laboratory experimental methods, where sampling was done randomly. Rats were grouped into 5 groups and each group consisted of 4 rats so that the animals test were obtained as many as 20 rats. Then the rats were grouped into several groups, i.e. positive control group (Captopril suspension in PGA 2% dose 4.5 mg/kg BW), negative control group (not tested, induced adrenaline in PGA 2% dose 1.2 mg/kg BB), instant granule preparation of roselle flower petals extract combination, squash and aloe vera leaves in 2% PGA with three dosage comparisons of 0.5 g/200 g BW, 0.75 g/200 g BW and 1 g/200 G BB.

4. Antihypertensive Effects Testing: Instant granules combinations of roselle extract, chayote squash and aloe vera were tested for their antihypertensive activity by the non-invasive method of Blood Pressure Volume Pressure Recording (VPR) using a CODA™ Non-Invasive Blood Pressure blood pressure meter with the following stages:

i) Early Blood Pressure Measurement: The initial blood pressure of the rat was measured using a Non-Invasive Blood Pressure CODA™ tool. The blood pressure obtained was recorded as normal blood pressure of the rat (BP I).

ii) Blood Pressure after Induced adrenaline Measurement: After normal blood pressure rats were recorded, the rats were induced with an adrenaline dose of 1.2 µg/kg BW administered intraperitoneally to achieve

hypertension state. Then the induced rat blood pressure was measured again after 30 minutes and the blood pressure obtained was recorded as high blood pressure or hypertensive blood pressure (BP II).

iii) Blood Pressure Measurement after Test Suspension: Blood pressure returned to normal for about 50-60 minutes after 30 minutes rats were induced with an adrenaline and the blood pressure obtained was recorded as high blood pressure or hypertensive blood pressure (BP II). Then, the rats were induced by the test suspension administered orally, then allowed for 15 minutes, after that the rats were induced with an adrenaline dose of 1.2 µg/kg BW administered intraperitoneally. After waiting for 30 minutes, blood pressure was measured again and recorded as final blood pressure (BP III).

iv) Antihypertensive Activity Calculation: Antihypertensive activity can be seen from the difference in the value of BP II - BP III. The antihypertensive activity of the test group was demonstrated by a percentage drop in blood pressure using the following formula:

$$\% \text{ Blood Pressure Lowering} = \left[\frac{(BP 2 - BP 3)}{BP 2} \right] \times 100\%$$

Annotation : BP 2 = Blood pressure after induced adrenaline (mmHg)

BP 3 = Blood pressure after given a dosage forms test (mmHg)

Data Analysis (ANAVA): The datas of percentage of rat's blood pressure decrease from the experiment was presented and analyzed using the design of Analysis of Variance (ANAVA) at the level of 0.05 and Newman-Keuls range test.

Results and Discussion

Extraction Results: The extraction of simplicia roselle petals is done by using maceration method. This method is performed to avoid damage to heat-resistant compounds because the anthocyanin pigment found in the flavonoid group in roselle flower petals is relatively unstable and flavonoids easily oxidize at high temperatures. In addition, maceration method is done because the process is relatively easy and the equipment used is simple.

Maseration is done by soaking the powder of roselle petals simplicia in 70% ethanol solvent for 3x24 hours, the solvent was replaced every 24 hours. After that, the liquid extract obtained was concentrated using a rotary evaporator at a temperature of 40 ° C. Then the extract is stored in the vapor plate and evaporated over the water bath until it weighs constant. The roselle flower petals thick extract obtained from 91.00 g of dried was 301.19 g, so the yield of the extract was 30.21%. Organoleptically, the extract of roselle flower petals has a thick consistency, dark red color, typical roselle odor and acidic taste.

To obtain the extract of chayote squashes and aloe vera leaves done by extortion and drying using freeze drier, this method is done to remove the water content from both plants so that the compounds contained in these plants can be extracted or soured by water during the process of extortion. Dried chayote squashes extract of 109.4 g of fresh plants of 7000 g obtained a yield of 1.56% while dried aloe vera extract obtained 34.84 g of fresh plants as much as 15,000 g and obtained a yield of 0.23%.

Result of Instant Granules Making: Based on the results of research conducted by Puspa et al,¹¹ a combination of roselle flower petals extract; chayote squash; and aloe vera leaves with a ratio of 240 doses; 250; 100 mg/kg BW has a systolic blood pressure decrease activity of 18.31% and diastolic was 23.19% better than single administration in male white rats. Rachmawati et al¹² have obtained instant granules formula with good quality in accordance with the requirements of granules. The formula can be seen in table 1.

Organoleptic observation of instant granules can be seen in table 2 and the results of evaluation of instant granule dosage form based on Rahmawati et al¹² can be seen in table 3.

Result of Antihypertensive Activity Test: This study aims to determine the dosage of instant granule dosage form of roselle flower petals, chayote squashes and aloe vera leaves that can provide optimal antihypertensive activity. The dose in this study was based on the results of a study conducted by Puspa et al¹¹ who said that the effective dose of combination of roselle flower petals extract; chayote squash; and aloe vera leaves respectively 240; 250; 100 mg/kg BB, then confirmed by Rahmawati et al¹² so that the instant granules formula obtained with different variations of the concentration of maltodextrin and sucrose that have good quality in accordance with the requirements of granules.

Based on these studies, the effective dosage of instant granules in sachet packaging (25g) is 0.5 g/200 g BB rat. The second and third doses are variations of the effective dose. The method used is the Non-Invasive Blood Pressure method with the CODA™ Kent Scientific Corporation blood tape measure against the white rats of the male *Rattus novergicus* Sprague dawley strain. The advantage of this method is not to provide pain in rats like other antihypertensive tests because no surgery was performed on rats.

In this study, each rat performed three times the blood pressure measurement of normal blood pressure (BP I), blood pressure after given test dosage form (BP II) and blood pressure after induced adrenaline (BP III). First, the measurement of blood pressure is normal. After the initial blood pressure measurement, perform intraperitoneal adrenaline induction to produce a state of hypertension.

Then the mouse is silenced for 50-60 minutes to eliminate the effects of hypertension so that blood pressure back to normal. Instant granule dosage forms made suspension with PGA 2 % and given to rats with dose variation of dose 1 (0.5 g/200 g BB), dose 2 (0.75 g/200 g BB) and dose 3 (1 g/200 g BB). Normal blood pressure measurement (BP I), blood pressure after adrenaline administration (BP II) and after administration of the dosage form test (BP III) are listed in table 4 and table 5.

Based on table 4 and table 5, we can see that the initial blood pressure measurement of systole has an average of 127.79 mmHg whereas the initial diastolic blood pressure has an average of 94.14 mmHg. Normal rat systolic blood pressure is 116-145 mmHg and hypertensive systolic blood pressure is > 145 to ≤ 200 mmHg¹⁵ whereas hypertensive rat diastolic blood pressure exceeds 90 mmHg¹⁶. It can be seen that the test results show the incidence of hypertension where the systolic blood pressure exceeds 145 mmHg. Test results show the incidence of hypertension where systolic blood pressure exceeds 145 mmHg and diastolic blood pressure exceeds 90 mmHg.

Systolic and diastolic blood pressure after administration of adrenaline in the 1st treatment group were greater than in the positive control group, dose 2, dose 3 and negative controls. After administration of the dosage forms test, the systolic blood pressure in the treatment group 1 was lower than in the positive control group, dose 2, dose 3 and negative control. While diastolic blood pressure after administration of test preparation in the treatment group dose 2 was lower than in the positive control treatment group, dose 1, dose 3 and negative control.

Furthermore, calculate the decrease in rat blood pressure and percentage decrease in rat blood pressure. The results of the calculation of blood pressure decrease and percentage decrease in blood pressure can be seen in table 6.

According to Thompson¹⁵, a substance test is said to have an antihypertensive effect if it can lower systolic blood pressure ≥ 20 mmHg. It is seen in table 5 that there is a decrease in systolic blood pressure ≥ 20 mmHg after administration of the dosage forms test. Then the test substance can be said to have antihypertensive effect. The result of the percentage of blood pressure drop activity after giving positive control, negative control, dose 1, dose 2 and dose 3 can be seen more succinctly in figure 1.

In figure 1, it can be seen that there is a blood pressure decrease each treatment group after instant granule dosage forms of positive control and negative control as standard. The activities of decreasing systolic and diastolic blood pressure were 1, 30,27% and 31,86%, 2, 21,77% and 21,27% respectively and 3 doses were 18,39% and 23,05%. The percentage of decreased blood pressure activity after

administration of adrenaline was greater in treatment group dose 1 (instant granule 0,5 g/200 g BW) compared with positive control group, dose 2 group, dose 3 group and negative control group. This suggests that treatment group dose 1 (instant granule 0.5 g/200 g BW) provided the highest antihypertensive activity compared with other treatment groups.

Data Analysis (ANAVA): Data of percentage of decreased activity of blood pressure were analyzed using variance analysis (ANAVA) to find out whether or not the influence of the test team in systolic and diastolic blood pressure technique was a real level of 0.05 (95% confidence). Statistical analysis was performed to determine whether or not the influence of the test group in systolic and diastolic blood pressure techniques. Back pressure variance analysis can be seen in tables 7 and 8.

The result of ANAVA of systolic and diastolic blood pressure shows that significance on both tables is 0.000 which means less than 0.05 then H_0 is rejected, it means with 95% confidence giving of instant granule dosage forms combination of roselle flower petals extract, chayote squash and aloe vera leaves have influence in lowering blood pressure both systole and diastole. Furthermore, to know which dose group has significant activity difference at 95% confidence, apply further test of Newman-Keuls. Further test results of Newman-Keuls can be seen in table 9 and 10 as follows.

Based on table 10, the results of this further test show that there is a statistically significant difference in the treatment given to the decrease in systolic blood pressure, in which dose group 3, dose 2 and positive control, differed significantly with negative control group and dose group 1 because spread of negative control group data and group dose 1 lies on different subset. Similarly, the dose group 1 had significant differences with the negative control group.

The dose 3 group did not show significant differences with the dose 2 group and the positive control group. This suggests that the dose 2 and dose 3 groups have the same effect of lowering the systolic blood pressure as the positive control group.

In table 10 it can be seen that statistically there are significant differences from the treatment group given to the decrease in diastolic blood pressure where the treatment group dose 2, positive control, dose 3 and dose 1 differed significantly with the negative control treatment group. This can be seen from the dissemination of different subsets in the negative control group. This suggests that there is a similar effect on dose 1, dose 2, dose 3 and positive controls in lowering diastolic blood pressure.

Table 1
The Instant Granul Formula of Rosela Flower Petals Extract, Chayote Squash and Aloe Vera Leaves (25g)

Composition	F(%)
Roselle extract	10.8
Chayote Squash extract	11.2
Aloe vera extract	4.48
Maltodextrin	48
Sucrose	25.44
Acesulfame	0.04
Benzoic acid	0.04

Table 2
Observation Result of Organoleptic Instant Granules

Organoleptic observation	Result
Color	Roselle
Odor	Typical roselle
Taste	Acid
Solubility	Easy soluble in water
pH	4.37

Table 3
Evaluation Result of Instant Granules

Evaluation Parameters of Instant Granules Dosage Form	Result
Break Angle (°)	11.3
Flow rate (gram/second)	7.28 ± 0.143
Water content (%)	2.67
Time dissolves (second)	30.27
Solubility speed (gram/ second)	0.661
pH	4.37

Table 4
Average Table of Systolic Blood Pressure Activity Rate of Each Treatment Group

Group	BP I (mmHg)	BP II (mmHg)	BP III (mmHg)
PC	126,64	158,49	121,32
D1	115,66	169,97	118,36
D2	139,57	155,82	121,88
D3	121,91	150,59	122,95
NC	135,17	163,57	157,81

Table 5
Average Table of Diastolic Blood Pressure Activity of Each Treatment Group

Group	BP I (mmHg)	BP II (mmHg)	BP III (mmHg)
PC	94,69	112,76	87,4
D1	83,67	129,83	88,15
D2	83,75	111,1	87,23
D3	89,70	111,36	85,70
NC	84,5	121,81	118,16

Table 6
Blood Pressure Activity and Blood Pressure Decrease Percentage after Positive Control, Negative Control, Dose 1, Dose 2 and Dose 3

No	Treatment Group	Decreased Blood Pressure of Rats			
		mmHg		%	
		Systolic	Diastolic	Systolic	Diastolic
1	KP	44,25	29,75	27,35	26,03
2		37,83	11,66	23,09	10,39
3		30	28,25	19,48	25,85
4		36,6	31,8	23,7	27,55
Average		37,17	25,36	23,4	22,45
1	D1	51,6	51,9	28,86	35,18
2		43,4	39,6	28,29	33,11
3		60,7	45,5	33,53	34,21
4		50,75	29,75	30,43	24,94
Average		51,61	41,68	30,27	31,86
1	D2	29,55	25,3	20,2	23,1
2		25,47	13,4	15,64	11,81
3		36,75	37	24,45	30,39
4		44	17,75	26,82	19,79
Average		33,94	23,36	21,77	21,27
1	D3	27,6	21,89	17,99	19,79
2		31	33,2	21,52	30,18
3		27,14	26,41	17,83	23,02
4		24,83	21,16	16,24	19,2
Average		27,64	25,66	18,39	23,05
1	KN	3,7	9,6	2,36	8,2
2		6,09	1,5	3,54	1,14
3		6,25	3,09	4,15	2,89
4		7	0,4	3,97	0,3
Average		5,76	3,64	3,50	3,13

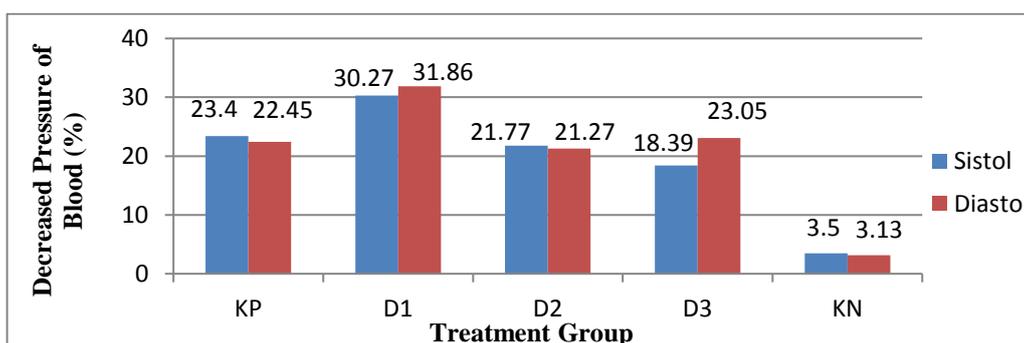


Fig. 1: The average of the percentages of blood pressure decrease (systolic and diastolic) blood of Sprague dawley male white rats after administration of an instant granule suspension combination of roselle flower petals extract, chayote squash and aloe vera leaves.

Table 7
Analysis of Variance (ANAVA) Percentage of Sistol Blood Pressure Reduction using IBM SPSS Statistics 20.

Variations Resource	JK	df	KT	F	Sig.
Correction Model	1574.240 ^a	4	393.560	42.958	0.000
Intercept	7587.748	1	7587.748	828.225	0.000
Treatment Group	1574.240	4	393.560	42.958	0.000
Error	137.422	15	9.161		
Total	9299.410	20			
Total Correction	1711.662	19			

Table 8

Analysis of Variance (ANAVA) Percentage of Sistol Blood Pressure Reduction using IBM SPSS Statistics 20.

Variations Resource	JK	df	KT	F	Sig.
Correction Model	1765.829 ^a	4	441.457	11.956	0.000
Intercept	8289.425	1	8289.425	224.500	0.000
Treatment Group	1765.829	4	441.457	11.956	0.000
Error	553.859	15	36.924		
Total	10609.113	20			
Total Correction	2319.687	19			

Table 9

Advanced Newman-Keuls Decreased Percentage of Systole Blood Pressure to Treatment Group using IBM SPSS Statistics 20.

Group	N	Subset		
		1	2	3
KN	4	3.5125		
D3	4		18.4014	
D2	4		21.7846	
KP	4		23.4083	
D1	4			30.2825
Sig.		1.000	0.081	1.000

Table 10

Advanced Newman-Keuls Decreased Percentage of Dyastole Blood Pressure to Treatment Group Using IBM SPSS Statistics 20.

Group	N	Subset	
		1	2
KN	4	3.1375	
D2	4		21.2778
KP	4		22.4624
D3	4		23.0514
D1	4		31.8637
Sig.		1.000	0.107

Conclusion

Instant granules of combination with roselle flower petals, chayote squash and aloe vera leaf at dose of 0.5 g/200 g BW have an optimal antihypertensive activity in male white rats with a percentage of systolic and diastolic blood pressure reduction activity of 30.27% and 31, 86%.

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