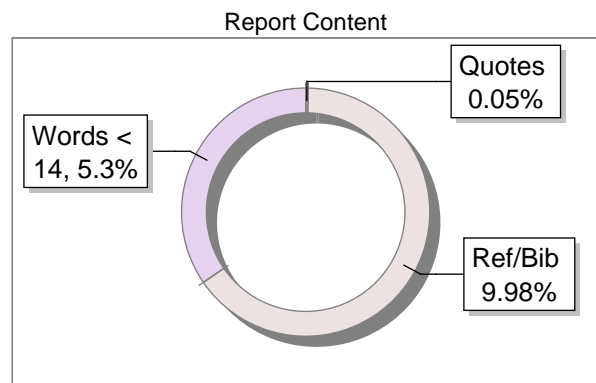
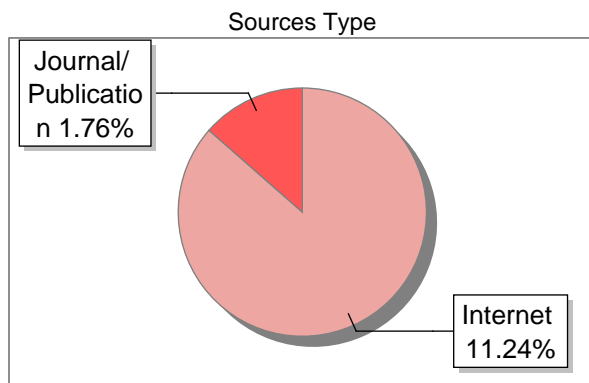
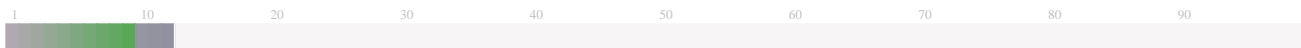


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26 Test Activity Combinations of Celery Herb (*Apium graveolens* L.) and Bay Leaf (*Syzygium polyanthum* W.) Ethanol Extract Toward Decreased Lipid Profile Levels in Hypertensive Mice

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Keywords: Hypertension, Hyperlipidemia, Celery Herb, Bay Leaf, Total Cholesterol, Triglycerides, LDL, HDL.

Abstract: Celery herb contains flavonoid which can hamper cholesterol synthesis through inhibitor HMG-CoA reductase. Bay leaf has a working mechanism that stimulates bile fluid secretion and stimulates blood circulation. This research aims to know the activity combinations of bay leaf ethanol extract (BLEE) and celery herb ethanol extract (CHEE) and to know which dose mostly can decrease lipid profile levels. The design of this research uses experimental with *pre-post control group design*. The mice are rendered with NaCl 8% solution and High Fat Feed. The mice are divided into 9 groups, in each group there are 5 mice which are normal group, control group, HCT group, Simvastatin group, and CHEE 4.50 mg/kgBW, BLEE 25.00 mg/kgBW, combination 1, combination 2, and combination 3. Data analysis is conducted with Normality test, *One Way ANOVA* test, *Kruskal-Wallis*, *Mann-Whitney*. Based on the research after they are induced with NaCl 8% solvent and given high-fat feed, the extract can increase total cholesterol levels, triglycerides, LDL, and also significantly decrease HDL ($p < 0.05$). After giving a test extract solution, it can reduce total cholesterol, triglycerides, LDL, and also significantly increase HDL levels ($p < 0.05$). The conclusion, the three variations combinations dose can decrease total cholesterol, Triglycerides, LDL, and also increase HDL levels, and combination dose 3 has the greatest decrease in total cholesterol, triglyceride, LDL, and the highest increase in HDL levels. So the lower the total cholesterol level, the lower the possibility of hypertension.

Introduction

Hypertension or high blood pressure can be defined as persistent blood pressure where the systolic pressure is above 140 mmHg and diastolic is above 90 mmHg. *World Health Organization* states that hypertension is a major cause of premature death worldwide, based on WHO data in 2012, 839 million people are suffered from hypertension and it is estimated will increase until reaches 1.5 billion in 2025 [1]. Hypertension is often called a *silent killer* because it turns out that many people do not realize they suffer from hypertension without knowing it over the years [2]. An unhealthy lifestyle; often consume food with plenty of salt, fat or high cholesterol food [3]. Lack of exercise can increase cholesterol levels within the body and cholesterol is a risk factor that can be changed from hypertension, so, the higher cholesterol level, the more risk to be exposed to hypertension [4]. Lipid profile (cholesterol total and triglycerides) has a significant relation with hypertension whereas HDL and LDL levels do not have significant relation with hypertension [5]. Rendering bay leaf extract is better in decreasing cholesterol level compared with giving statin drug [6].

Bay leaf ethanol extract has activity in decreasing cholesterol level by hampering HMG-CoA reductase activity [7]. Herbal therapy on celery that contains substances which can decrease blood pressure such as; apiin and mannitol, a diuretic trait which helps the kidneys remove excess fluid and salt from the body [8]. Celery herb extract can also decrease atherosclerosis risk of the tested animal by fixing lipid profile blood which induced with hyperlipidemia [9]. Based on the preliminary test,

rendering 4.5 mg/kgBW of celery herb extract can reduce blood pressure and rendering 25 mg/kgBW of bay leaf extract can decrease cholesterol levels. Based on that background, the research was conducted by combining celery herb ethanol extract and bay leaf ethanol extract in mice induced with NaCl solution and high-fat feed, so, it is expected that the combination is better in decreasing cholesterol levels, triglycerides, LDL, and increasing HDL levels in the blood.

Material and Methods

Plant materials. Celery herb and bay leaf were obtained from the Beringharjo market, Yogyakarta and identified in Biology Laboratory of Ahmad Dahlan University. In the process ethanol extract of celery herb and bay leaf using filtering results method with ratio sample and the solvent was 1:10 (b/v). Then, the powder was soaked using ethanol 70% for the first 6 hours while it stirred and then kept it still for 18 hours. Filtering results separated from the pulp and used filter paper to the filtrate until it was obtained celery herb and bay leaves liquid extract. The following step was conducted maceration for 24 hours (ratio 1:1). Afterwards, it was conducted separation between filtering results and the pulp. Combining the first filtering results and the filtering results of liquid extracts then evaporated using an evaporator to obtain a thick extract of celery herb and bay leaf [10].

Animals. Tested animals used for the research was male furrow Wistar mice of 2-3 months old, weighing 150-250 gram, in the amount of 45 mice. Tested animals were placed in constant temperature ($22 \pm 2^\circ \text{C}$) with relative constant moisture ($55 \pm 10\%$), and had free access to get food and water. Animal protocol handling in the research was adopted by animal handling manual from Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Ahmad Dahlan. All studies were approved by the Research Ethics Committee Ahmad Dahlan University (Ethical Approval Number 011805079).

Experimental Design

The mice were divided into nine groups, in each of the groups contained 5 mice. The treatment was conducted for 22 days. Distribution volume in mice was 2ml/200g mice.

Normal group: were provided with food and water for 21 days. Control group: tested animals were given NaCl 8% solution and high-fat feed (14 days) and rendered CMC-Na 0.5% (7 days). HCT group: tested animals were given NaCl 8% solution and high-fat feed (14 days) and rendered hydrochlorothiazide suspense solution 2.25 mg/kgBW mice (7 days). Simvastatin group: tested animals were given NaCl 8% solution and high-fat feed (14 days) and rendered simvastatin suspense solution 0.9 mg/kgBW (7 days).

Extract comparison group: tested animals were supplied with NaCl 8% and high-fat feed (14 days). CHEE group: were supplied with celery herb ethanol extract (CHEE) 4.5 mg/kgBW mouse (7 days). BLEE group: were supplied with bay leaf ethanol extract (BLEE) 25 mg/kgBW mouse (7 days). Combination dose group: tested animals were supplied with NaCl 8% solution and high-fat feed (14 days). Combination group 1: was supplied with CHEE 1.125 mg/kgBW mouse and BLEE 6.25 mg/kgBW mouse. Combination group 2: was supplied with CHEE 2.25 mg/kgBW mouse and BLEE 12.5 mg/kgBW mouse. Combination group 3: was supplied with CHEE 4.5 mg/kgBW mouse and BLEE 25 mg/kgBW mouse.

Lipid Profile Level Measurement. Lipid profile level measurement was conducted in day-0, 15, and 22. The measurement used mouse serum which was taken through the eye using a capillary pipe. The measured lipid profile level was total of cholesterol, triglycerides, HDL and LDL. Total cholesterol level measurement using the CHOD-PAP method, triglycerides level measurement using the CHOD-PAP method, HDL level measurement using precipitation which will be measured using CHOD-PAD method, and LDL level measurement using Friedewald formula.

Statistic Analysis. Data obtained was analyzed using SPSS 16 software. The analyzed test was a homogeneity test and normal distribution test. To see the relationship between groups, one-way analysis of variance tests (ANOVA) was conducted, if there was a significant difference, the Least Significant Difference (LSD) test was performed. *Kruskal-Wallis* non-parametric analysis test was

conducted if the data were not normally distributed and homogeneous. If there were differences, then proceed with *the Mann-Whitney* non-parametric analysis test.

Result and Discussion

Extraction Result. The result of celery herb extraction is thick extract as much as 177.19 grams from the total powder of 2.60 kg, so it obtained 6.82% yield. The result of bay leaf extraction is 112.44 grams from a total of 2.20 kg of powder, so it obtained 5.11% yield

Lipid Profile Measurement Result. Total Cholesterol Level. Total cholesterol level measurement in the 15th day shows enhancement compared with day-0, cholesterol level in group normal is lower compared with the other group, which is 118.94 mg/dL because it was not supplied with high-fat feed. Single-dose group BLEE 25 mg/kgBW and combination 3 show that there are significant differences with the control group. The day-22 shows there is significant decrease where all group are significantly different from the control group. All group are significantly different ($p < 0.05$) with the normal group. Average \pm SD can be seen in Table 1.

Simvastatin group has the biggest percentage of decrease with 67.47 %. Extract treatment with a single dose, bay ethanol extract decreases cholesterol level much greater than celery herb ethanol extract, which is 44.57 %. The result of cholesterol level for extract combination dose, combination 3 with combination ethanol extract of celery herb 4.5 mg/kgBW and bay leaf 25 mg/kgBW has greater decrease, which is 47.25 % compared with the other two combination groups. Combination dose 2 with a combination of CHEE 2.25 mg/kgBW and BLEE 12.50 mg/kgBW has the smallest percentage of the decrease compared with other groups, which is 13.98 %.

Table 1. Total Cholesterol Level

Group	Dose (mg/kgBW)	Average \pm SD (mg/dL)			Decreasing Percentage
		Day 0	Day 14	Day 22	
Normal		114.27 \pm 1.24	118.94 \pm 2.78*	117.23 \pm 5.71 *	1.46*
Control		114.75 \pm 2.78	182.44 \pm 4.50	166.33 \pm 2.07	9.68
HCT	2.25	115.34 \pm 2.58	173.86 \pm 12.25	147.33 \pm 7.07*	18.01
Simvastatin	0.9	113.67 \pm 2.69	174.65 \pm 6.31	104.29 \pm 5.92*	67.47*
CHEE	4.5	114.27 \pm 2.09	172.67 \pm 3.08*	131.75 \pm 9.55*	31.06*
BLEE	25	112.24 \pm 1.58	182.84 \pm 3.93	126.47 \pm 7.98*	44.51*
Combination 1	CHEE 1.125: BLEE 6.25	115.58 \pm 2.48	182.57 \pm 6.34	153.93 \pm 4.75*	18.61
Combination 2	CHEE 2.25: BLEE 12.5	115.34 \pm 2.00	168.98 \pm 15.26	148.25 \pm 8.92*	13.98
Combination 3	CHEE 4.5: BLEE 25	114.50 \pm 3.94	169.50 \pm 7.21	115.12 \pm 6.74*	47.25*

Note: * $p < 0.05$ significantly different from Control group

CHEE: celery herb ethanol extract

BLEE: bay leaf ethanol extract

Triglycerides Level. The result of triglycerides level measurement in the 15th day shows an increase compared with day-0. Triglycerides level in the normal group is lower compared with the other group which is 117.11 mg/dL. The day-22 shows significant decrease where all of the groups are significantly different from the control group. The 15th day, all groups are significantly different from the normal group. The 22nd day, HCT group, CHEE 4.5 mg/kgBW, combination 1, combination 2, and combination 2 are significantly different ($p < 0.05$) with the normal group, however, for triglycerides level value is almost the same with the normal group. Average levels \pm SD can be seen in Table 2.

HCT group can decrease triglycerides as much as 16.24 %. Simvastatin group has the greatest decreasing percentage by 29.85 % compared with HCT, because HCT is included in hypertension

medicine thiazide-type. Extract single dose extract group, BLEE dose 25 mg/kgBW has greater decreasing percentage than CHEE 4.5 mg/kgBW which is 19.73%. Extract combination dose group has the greatest decreasing percentage compared with all of the other groups which are 37.41%. Combination 1 with combinations of CHEE 1.13 mg/kgBW and BLEE 6.25 mg/kgBW has the smallest decreasing percentage compared with the others, which is 14.99%.

Table 2. Triglycerides Level

Group	Dose (mg/kgBW)	Average \pm SD (mg/dL)			Decreasing Percentage
		Day-0	Day-15	Day-22	
Normal		113.00 \pm 3.34	117.11 \pm 7.98*	116.65 \pm 8.13*	0.39
Control		115.29 \pm 2.56	159.45 \pm 1.05	149.76 \pm 3.78	6.08
HCT	2.25	118.02 \pm 1.97	158.34 \pm 1.33	132.62 \pm 14.73*	16.24
Simvastatin	0.9	113.31 \pm 2.23	158.48 \pm 3.82	111.18 \pm 7.40*	29.85*
CHEE	4.5	112.24 \pm 7.48	160.28 \pm 2.52	132.02 \pm 6.49*	17.63*
BLEE	25	117.87 \pm 3.18	156.13 \pm 4.45	125.32 \pm 13.83*	19.73*
Combination 1	CHEE 1.125: BLEE 6.25	116.50 \pm 4.04	152.25 \pm 6.69	129.43 \pm 9.66*	14.99
Combination 2	CHEE 2.25: BLEE 12.5	113.00 \pm 2.12	160.15 \pm 3.71	130.49 \pm 11.79*	18.52*
Combination 3	CHEE 4.5: BLEE 25	114.24 \pm 2.46	166.69 \pm 9.24	104.34 \pm 5.75*	37.41*

Note: *p < 0.05 significantly different from Control group
 CHEE: celery herb ethanol extract
 BLEE: bay leaf ethanol extract

HDL Level. HDL level measurement on the day-15, occurred HDL level decreasing compared with day-0, except in normal group which is 55.12 %. On the day-22, it is occurred HDL level increase compared with day-15. The statistic test result, day-0 for BLEE 25 mg/kgBW and combination 3 are significantly different from the control group. All of the groups are significantly different with the control group, but they have greater HDL level than the normal group and all of the groups also have a different average ($p < 0.05$) with the normal group in the day-15. Whereas on the day-22, all of the groups also show significant difference with the control group. Simvastatin group, combination 2 and combination 3 have different average with the normal group; however, the HDL level is almost the same with the normal group. Average \pm SD can be seen in Table 3.

Simvastatin can increase HDL level greater than HCT with an increased percent of 41.80% because HCT is hypertension medicine thiazide-type. The single biggest dose of extract increasing HDL levels is BLEE 25 mg / kgBW with an increased percent of 41.99%, combination dose 3 posses the biggest percent increase among other groups which is 62.03%.

Table 3. HDL Level

Group	Dose (mg/kgBW)	Average \pm SD (mg/dL)			Increasing Percentage
		Day 0	Day 15	Day 22	
Normal		62.96 \pm 1.25	55.12 \pm 1.57*	57.87 \pm 4.08*	4.99*
Control		63.78 \pm 1.13	49.49 \pm 3.19	42.47 \pm 3.42	-14.17
HCT	2.25	64.88 \pm 1.66	43.71 \pm 1.04*	56.63 \pm 3.49*	29.56*
Simvastatin	0.9	62.41 \pm 2.64	44.40 \pm 2.15 *	62.96 \pm 1.15*	41.80*
CHEE	4.5	62.68 \pm 2.14	44.40 \pm 3.32*	57.60 \pm 3.75*	29.72*
BLEE	25	61.03 \pm 1.23*	42.89 \pm 2.26*	60.89 \pm 2.86*	41.99*
Combination 1	CHEE 1.125: BLEE 6.25	62.13 \pm 1.79	45.36 \pm 1.75*	55.67 \pm 2.12*	22.73*
Combination 2	CHEE 2.25: BLEE 12.5	62.13 \pm 2.04	45.09 \pm 4.00*	61.86 \pm 5.17*	37.20*
Combination 3	CHEE 4.5: BLEE 25	61.31 \pm 2.09*	43.44 \pm 2.09*	70.38 \pm 1.58*	62.03*

Note: *p < 0.05 significantly different from Control group
 CHEE: celery herb ethanol extract
 BLEE: bay leaf ethanol extract

LDL Level. LDL level measurement on the day-15, occurred LDL level increase compared with the day-0, except for normal group which is 40.40 mg/dL. LDL level on the day-22 decrease compared with the day-15. The result statistic test, day-15 for group BLEE 25 mg/kgBW is significantly different with the negative control group and all of the groups are different with the normal group, however, LDL level is almost the same with the normal group. Average \pm SD LDL level can be seen in Table 4.

Simvastatin group can decrease LDL Level as much as 80.62%, whereas for HCT group can decrease as much as 34.84% because HCT is hypertension medicine, thiazide group. Extract single-dose group, BLEE 25.00 mg/kgBW can decrease bigger than CHEE 4.50 mg/kgBW which is 62.74%. Combination 1 can decrease LDL level for 32.22% and combination 2 can decrease as much as 34.36%.

Table 4. LDL Level

Group	Dose (mg/kgBW)	Average \pm SD (mg/dL)			Decreasing Percentage
		Day 0	Day 15	Day 22	
Normal		28.71 \pm 1.91	40.40 \pm 3.55*	36.03 \pm 9.06*	10.83
Control		27.91 \pm 2.76	101.07 \pm 4.39	93.91 \pm 3.25	7.082
HCT	2.25	26.86 \pm 3.52	98.46 \pm 12.75	64.17 \pm 10.18*	34.84*
Simvastatin	0.9	28.61 \pm 2.95	98.56 \pm 4.08	19.01 \pm 7.55*	80.62*
CHEE	4.5	29.14 \pm 2.11	96.23 \pm 2.66	47.75 \pm 8.91*	50.37*
BLEE	25	27.63 \pm 2.76	108.73 \pm 3.95	40.51 \pm 8.14*	62.74*
Combination 1	CHEE 1.125: BLEE 6.25	30.15 \pm 2.58	106.76 \pm 8.68	72.37 \pm 4.38*	32.22*
Combination 2	CHEE 2.25: BLEE 12.5	30.61 \pm 2.31	91.86 \pm 15.67	60.30 \pm 12.72*	34.36*
Combination 3	CHEE 4.5: BLEE 25	30.35 \pm 5.67	92.73 \pm 9.46	23.87 \pm 5.93*	74.26*

Note: *p < 0.05 significantly different from Control group

CHEE: celery herb ethanol extract

BLEE: bay leaf ethanol extract

The research was conducted using hypertension mice which were induced NaCl 8% solution and supplied with high-fat feed for 14 days, where the blood pressure exceed 150/90 mmHg. High-fat feed composition was the mixture of goat fat (10%) and quail egg yolk (5%) with standard feed. Supply of high-fat feed for 14 days could increase lipid profile levels in the blood [11]. Supplying high-fat feed also could increase the activity of hepatic lipase which resulted in HDL level decreased and also reduced HDL size and increased LDL [12]. LDL is easily oxidized, so that, it can form foam cell which can tie each other. Thus, it is created a lump in the form of the bump which can stick to the vein. This can cause the veins to become constricted dan the bloodstream is clogged and then blood is pushed harder, on the consequence, the blood pressure becomes high.

The body in normal condition can maintain cholesterol balance through an inhibition feedback mechanism by cholesterol cell hampering biosynthesis new cholesterol. Cholesterol level in the blood is increasing, then it would hamper its biosynthesis by hampering enzyme activity β -hydroxyl methyl glutaric-Ko-A (HMG-KoA) reductase that catalyzes mevalonate formation of HMG-KoA. Excessive high-fat feed intake cause HMG-KoA enzyme can not function optimally, so that, the fat surplus will still become fat and stored within adipose tissue [13].

Extract treatment uses celery herb and bay leaf ethanol extract. Saputra research *et al.* (2016), shows that celery herb contains apigenin which can prevent blood vessels constrict. Bay leaf ethanol extract has a working mechanism of stimulating bile fluid secretion, so that, cholesterol will come out along with bile fluid through the intestine and stimulate blood circulation, thus reducing the occurrence of deposition of fat in blood vessels [8].

Cholesterol is a hypertension risk factor which can be changed, so the higher the total cholesterol level, the higher the possibility of hypertension [2]. Cholesterol accumulation in the blood vessels wall will decrease blood vessels permeability that can increase blood pressure and cause hypertension [3].

HDL works to clean cholesterol from the walls of blood vessels by transporting it back to the liver so it is called "good" cholesterol. Main protein that formed HDL is Apo-A [15]. The main function is as a place to store Apo-C and Apo-E that is needed in Kilomicron metabolism and VLDL. HDL is also called good cholesterol. HDL carries free cholesterol located in endothelial peripheral tissue including blood vessel to HDL receptor in the liver, it is formed into bile and excreted to the small intestine to help digest fat process that will be excreted in the form of faeces. The increasing of triacylglycerol level makes the VLDL and IDL formation increase, so that, LDL which comes from IDL also increase and cause disorder in storage balance and carrying cholesterol peripheral tissue. The increasing dietary cholesterol storage (Exogen cholesterol) in peripheral tissue leads to HDL concentration decrease that has the role in inducing cholesterol excretion from peripheral tissue [16]. LDL contains fat larger than HDL. The main protein is apolipoprotein B (Apo B). LDL also induces cholesterol sticking in the blood vessel wall so that it called "bad" cholesterol". LDL is produced in the liver, LDL and enters into blood circulation filters cholesterol which circulates in the blood. The more cholesterol LDL receptor in the liver, the higher filtering level conducted, so, LDL cholesterol in the blood is low. The deficiency of cholesterol LDL receptor in the liver will increase cholesterol LDL level in the blood. The higher cholesterol LDL level can lead to the process of heart muscle death. LDL enters to arteries blood wall, it will appear *plaque* in which it collides with protein, and afterwards, it will cover by muscle cells and calcium. In the long term, it will lead to atherosclerosis (stiffening and constricting of blood vessels). So that, oxygen supply and nutrition to all over the body is hampered that can lead to heart attack, stroke and other disorder [15].

Conclusion

Rendering celery herb ethanol extract combination (*Apium graveolens* L) and bay leaf ethanol extract (*Syzygium polyanthum*) with combination dose as follows: CHEE 1.13 mg/kgBW and BLEE 6.25 mg/kgBW; CHEE 2.25 mg/kgBW and BLEE 12.50 mg/kgBW; CHEE 4.50 mg/kgBW and BLEE 25.00 mg/kgBW is able to decrease total cholesterol level, triglycerides, and LDL and also can increase HDL level.

Combination dose which can decrease cholesterol level, triglycerides level, LDL level and increase HDL level on lipid profile level is the combination 3 (CHEE 4.50 mg/kgBW and BLEE 25.00 mg/kgBW) and this combination better than using bay leaf or celery herb individually. The implication of this combination is able to decrease on lipid profile, so will increase blood vessels permeability that can decrease blood pressure and cause hypertension.

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