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The Potential of African Leaf Extract *(Gymnanthemum amygdalinum Del.)* as Antihypertensive in Male White Rats

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Abstract. Hypertension is a non-communicable disease that often occurs in the community and causes serious health problems. The number of cases of hypertension that occurs in Indonesia increases the exploration of the use of natural ingredients, one of which is African leaves as a traditional medicine that is developed into herbal medicine and standardized herbal medicine. Flavonoids are known to have the ability to inhibit the activity of the angiotensin-converting enzyme. African leaves are one of the plants that contain flavonoid secondary metabolites that can be obtained by the MAE (Microwave Assisted Extraction) extraction method using 70% ethanol as a solvent. Induction of hypertension was carried out by giving 2% prednisone-NaCl solution for 21 days, the positive control used was the ACEI captopril group with a dose of 0.45 mg/200 g BW and the preparation of African leaf extract was made in 3 doses, namely the dose 100 mg.kg-^{1} BW, dose II 150 mg.kg^{-1} BW, and dose III 200 mg.kg^{-1} BW) given orally. Blood pressure was measured every 7 days using a CODA® non-invasive blood pressure measuring device. The results showed that the African leaf has an antihypertensive effect where the most effective dose in lowering blood pressure is dose III 200 mg.kg^{-1} BW with a blood pressure reduction time of 14 days.

Keywords: antihypertension; african leaf; Microwave Assisted Extraction (MAE); angiotensin-converting enzyme (ACE)

INTRODUCTION

Hypertension or better known as high blood pressure is still a disease that is often attached to the elderly and even occurs in adolescents and children. The prevalence of hypertension that occurs in Indonesia based on doctor's diagnosis has decreased from 9.4% (2013) to 8.4% (2018), while based on the results of measurements in the population aged 18 years there has been an increase in prevalence from 25.8% (2013) to 34.1% (2018) [1].

Estimated cases of hypertension in 2018 reached 63 million people and caused more than 400 thousand deaths due to hypertension [2]. Globally, it is estimated that 26% (972 million people) of the world's population have a history of hypertension, and its prevalence in 2025 is expected to increase.

Indonesia has approximately 30,000 species of plants, with at least 9,600 species of plants known to be efficacious as medicinal plants and approximately 300 species that have only been used as ingredients for traditional medicine [3]. African leaves are empirically used by the community as a support for the treatment of a disease.

African leaf studies have been carried out such as being able to reduce cholesterol levels starting from a dose of 100 mg.kg^{-1} BW, 150 mg.kg^{-1} BW to 200 mg.kg^{-1} BW and have an effect on reducing uric acid levels with a concentration of 0.9% w/v [4]. From the various studies that have been carried out, there has been no research on the effect of African leaf extract as an antihypertensive. Flavonoids have the ability to inhibit the activity of the angiotensin-converting enzyme. African leaves are reported to have a total flavonoid content of 1.27% [5].

MATERIALS AND METHODS

Tools

The tools used are aluminum foil (Klin Pak®), measuring flask (Pyrex®), porcelain cup, funnel (Pyrex®), disposable syringe (OneMed Healthcare), grinder (Panasonic®), hair dryer (Philips), mesh 40, analytical balance (LabPRO DT224C®), Erlenmeyer (pyrex®), measuring cup (pyrex®), filter paper, vacuum rotary evaporator, experimental animal scales, metabolic cage, sonde, CODA® blood pressure measuring device, thermometer, oven, crucible, and furnace.

Materials

The materials used are distilled water, hydrochloric acid (HCl), captopril®, african leaves, 70% ethanol (CH_2OH), gelatin, sodium chloride (NaCl), Buchardat reagent, Dragendorff reagent, Mayer reagent, magnesium powder (Mg), iron III chloride ($FeCl_3$), zinc (zn) powder, standard feed, prednisone®, Sprague-Dawley male rat.

Methods

African Leaf Extraction

African leaf powder was extracted using the MAE (Microwave Assisted Extraction) method by inserting the African leaf powder into an Erlenmeyer and added with 70% ethanol solvent at 1:10. Extraction was carried out using the optimization results of the MAE method according to [6] with high power 600 watts using 70% ethanol solvent in 4 minutes. Periodically irradiated (1 minute irradiated and 2 minutes turned off) to keep the temperature from rising above 800°C. Then allowed to stand until it reaches room temperature, then filtered and the volume of filtrate obtained is calculated, then stored in a brown bottle. The filtrate obtained was evaporated using a vacuum rotary evaporator to form a thick extract. The extract obtained was calculated in terms of yield and then stored in a tightly closed container and protected from sunlight.

Phytochemical Analysis

Flavonoid Test

Identification of flavonoids was carried out by adding 2 mL of the extract solution with Mg powder and adding 10 drops of 2N HCl and then shaking. Judging from the color change, the presence of orange to red color indicates the presence of flavonoid compounds [7].

Alkaloid Test

Identification of alkaloids was carried out by means of 3 mL of the extract solution added with 2N sulfuric acid while stirring, then tested using 3 alkaloid reagents namely Dragendorff's reagent, Buchardat's reagent and Mayer's reagent. the formation of a red precipitate in the Dragendorff reagent while the Mayer reagent formed a yellowish white precipitate, and the Buchardat reagent formed a brown to black precipitate indicating the presence of flavonoid compounds [7].

Tannin Test

The identification of tannins was done by adding 1 mL of the extract solution with 10% FeCl3. The presence of a dark blue or greenish black color indicates the presence of tannin compounds [7].

Saponin Test

Saponin identification was carried out by adding sufficient water to the extract. Then shaken vertically for 10 minutes. The presence of foam indicates the presence of saponin compounds [7].

Quality Characteristics

Determination of Ash Level

Determination of ash content in simplicia was carried out by carefully weighing as much as 2 grams of thick extract, then put into a porcelain crucible that had been tared, then ignited until the charcoal ran out and then cooled, then weighed. The ash content is calculated on the material that has been dried in the air [8] ash content formula:

$$\%\ \text{Ash level} = \frac{(W.krus + ash) - (W.\ empty\ krus)}{W.\ extract} x100\%$$

Determination of Water Level

Determination of water content is done by gravimetric method. In this way, 2 grams of African leaf extract were weighed, dried at 105°C to a constant weight, with a weight deviation of 0.25% and then cooled. After cooling, the cup containing the sample was weighed [8]. Water level formula:

$$\%\ \text{Water Level} = \frac{W_0 - W_1}{\text{W sample}}\ x\ 100\ \%$$

Experimental Animal Treatment

Hypertension Induction in Experimental Animals

The experimental animals used in this study were 30 male white rats of the Sprague-Dawley strain weighing 150 - 200 grams. In this study, 6 replicates were used. After acclimatization for 7 days, the test animals were calculated CV and measured initial blood pressure, then continued with hypertension induction using a combination of prednisone and 2% NaCl orally for 21 days. Rats were categorized as hypertensive if their systolic blood pressure was more than 140 mmHg and diastolic blood pressure was 90 mmHg. All treatments with experimental animals in this study have obtained ethical clearance from Animal Use Committee Faculty of Mathematics and Natural Science, Universitas Pakuan number 024/KEPHP-UNPAK/10-2021.

Antihypertensive Test on Experimental Animals

African leaf extract was given to mice every day for 14 days. Administration was done orally using a probe in accordance with 3 dose groups, namely 100, 150 and 200 $mg.kg^{-1}$ BW. The positive control group used captopril® with the same time intervention. After 7 days of administration, the rats blood pressure was measured again.

Blood Pressure Measurement

Blood pressure measurement was carried out using a *non-invasive blood pressure measuring device.* This method is a *non-invasive method* or does not require surgery using a CODA® device. The non-invasive blood pressure measurement method was carried out by placing the rat in the animal holder with the tail hanging outward, then measuring the temperature of the rat first (32-35°C). Then the tail is clamped with a pressure kit in the form of VPR and O-CUFF and then connected to a pressuremeter to determine systolic and diastolic blood pressure.

Data Analysis

Data obtained from results measurement pressure blood systolic and diastolic with mmHg unit. Data analysis used the IBM SPSS *Statistics 24 for windows program* with ANOVA (*Analysis of variance) test* using a factorial RBD (Randomized Block Design) design pattern which was then followed by Duncan's test.

RESULTS AND DISCUSSIONS

Results of African Leaf Extraction

The results of the thick extract of African leaves obtained from extraction with the MAE method were 29,173 grams from 200 grams of African leaf simplicia powder that had been extracted. The yield obtained is 14.586%.

Characteristic Quality Test Results

Ash Level Test Results

The principle of ash content is the ashing of all organic substances at high temperatures which are then weighed after the ashing process. The higher the ash content obtained, the higher the mineral content in a material. The results of the ash content test obtained are not much different from the results of the ash level test in [9] which is 2.37% and the results both meet the quality requirements according to [3] African leaves have an ash content of <10.2%. The results of the ash level test can be seen in Table 1.

TABLE 1. Average Result of Ash Level

Average Ash Level	
Powder	Extract
4.452%	5.003%

Water Level Test Results

The principle of content of gravimetry is based on weighing the amount of water that is not bound by a material with a weight deviation of 0.25% after heating the oven at a temperature of 105°C. The results of the water content test obtained are not much different from the results of the ash level test in the [10] which is 3.90% and the results both meet the quality requirements according to [3]. African leaves have a water content of < 12.5%. Calculation of water content can be seen in Table 2.

TABLE 2. Results Average Water Level

Average Water Level	
Powder	Extract
8.602%	4.199%

Phytochemical Test Results

Phytochemical tests were carried out in this study in the form of tests for flavonoid compounds, alkaloids, saponins, and tannins. Phytochemical test is a test that aims to determine the content of secondary metabolites in a material. The results of the phytochemical test of simplicia powder and African leaf extract showed that both contained flavonoid compounds, alkaloids, tannins and saponins. This is also supported by research by [11] where positive African leaves contain secondary metabolites of flavonoids, alkaloids, tannins, and saponins. Phytochemical test results can be seen in Table 3.

TABLE 3. Phytochemical Test Results

Compound Identification	Powder	Extract	Parameter
Flavonoids			
- Mg	+	+	Orange
- Zn	+	+	Orange
Alkaloids			
- Buchard	+	+	Dark chocolate
- Dragendroff	+	+	Orange brown
- Mayer	+	+	white
Tannins			
- $FeCl_3$	+	+	Green/Dark Blue
- Gelatin	+	+	White
- Gelatin + NaCl	+	+	White
Saponins	+	+	Foam does not disappear

Results of Induction in Experimental Animals

After being acclimatized for 7 days, the experimental animals were weighed to calculate the coefficient of variance (CV) which aims to see the homogeneity of the body weight of the experimental animals used in the treatment, the CV obtained was 10.27%, the CV obtained met the requirements, namely <15% [12]. The induction method used was DOCA-Salt in the form of Prednisone-NaCl2% which was administered daily for 21 days orally which was then measured every 7 days. Induction aims to increase the blood pressure of experimental animals before treatment so that the effects of the plants used in the study can be seen. The increase in blood pressure in experimental animals was due to the induction of DOCA-Salt. DOCA-Salt is one model of hypertension caused by endocrine (hormonal) influences. According to [13]. Deoxycorticosterone is a steroid hormone produced by the adrenal glands which has activity as a mineralocorticoid. Prednisone is a corticosteroid drug that has side effects in the form of mineralocoticoids. Mineralocoticoids work by retention of Na+ ions and excretion of K+ ions. Data on the results of systolic and diastolic blood pressure measurements can be seen in Table 4.

TABLE 4. Results of Increased Blood Pressure After Induction

Group	**Before Induction (mmHg)**		**After Induction (mmHg)**		**Percentage Increase (%)**	
	systolic	**diastolic**	**systolic**	**diastolic**	**systolic**	**diastolic**
Negative (Aquades)	118.00	81.80	170,40	117.20	44.67	43.27
Positive (Captopril®)	112,20	84.00	164.81	116.60	46.88	38,80
Dosage 100 mg	113.20	83.33	167.80	116.80	47.71	38.96
Dosage 150 mg	113.60	85.60	167.60	115.00	47.53	34.34
Dosage 200 mg	110,40	80,17	166,40	113.20	50.45	40.44
$\overline{x}$	113.48 ± 2.70	82.98±1.86	167.40 ± 1.83	115.76 ± 1.48	47.45	39.16

Antihypertensive Test Results in Experimental Animals

After an increase in blood pressure and stability on the 21st day, the experimental animals were then given healing treatment which was divided into 5 treatment groups, namely a negative control group in the form of distilled water, a positive control group in the form of captopril, and 3 dose groups consisting of a dose of I 100 mg/day. kgbw, 150 mg/kgbw and 200 mg/kgbw of african leaf extract in each group there were 5 experimental animals and were given treatment with a volume of 1 ml for each experimental animal. Systolic and diastolic blood pressure was measured every 7 days using a CODA blood pressure measuring device. The results of the average and standard deviation of systolic and diastolic blood pressure measurements each week after treatment on experimental animals can be seen in Table 5 and Table 6.

TABLE 5. Results of Average Systolic Blood Pressure Decrease

Group	Average Systolic Blood Pressure Decrease		
	H0	H7	H14
Negative (Aquades)	170.41 ± 9.06 ^{d}	169.80 ± 6.10 ^{d}	169.07 ± 4.09 ^{d}
Positive (Captopril®)	164.81 ± 4.51 ^{cd}	152.25 ± 5.78 ^{b}	125.50 ± 3.44 ^{a}
Dosage 100 mg	167.80 ± 4.95 ^{d}	157.80 ± 2.85 ^{bc}	133.20 ± 5.70 ^{a}
Dosage 150 mg	167.60 ± 2.87 ^{d}	156.00 ± 4.33 ^{bc}	130.80 ± 6.96 ^{a}
Dosage 200 mg	166.40 ± 5.60 ^{d}	155.60 ± 4.27 ^{bc}	128.40 ± 8.01 ^{a}

Description: numbers marked with the same superscript letter in the same row or column show no difference

TABLE 6. Average Result of Lowering Diastolic Blood Pressure

Group	Average Decrease in Diastolic Blood Pressure		
	H0	H7	H14
Negative	117.21 ± 8.70 ^{f}	116.40 ± 9.30 ^{f}	116.00 ± 4.70 ^{f}
Positive	116.60 ± 5.42 ^{f}	94.80 ± 4.95 ^{bc}	83.64 ± 6.01 ^{a}
Dosage 100 mg	116.81 ± 5.00 ^{f}	107.42 ± 7.40 ^{def}	91.40 ± 6.25 ^{ab}
Dosage 150 mg	115.00 ± 4.97 ^{f}	104.60 ± 5.71 ^{de}	89.80 ± 6.10 ^{ab}
Dosage 200 mg	113.20 ± 3.70 ^{ef}	102.0 ± 6.72 ^{cd}	88.40 ± 6.88 ^{ab}

In Table 5 and Table 6, it can be seen that there was a decrease that occurred every week after administration of African leaf extract, both systolic blood pressure and diastolic blood pressure. The results of the ANOVA statistical test using the RAK (Randomized Block Design) method of actorial pattern showed that the effect of the group, the time of administration and the interaction between the groups and the time of administration on blood pressure had a significant effect because it obtained a significant value of 0.000 where the sig obtained was <0.05 so proceed with Duncan's test.

The results of Duncan's further test showed that the positive control group had the same effect as the treatment of African leaf extract in all dose groups on reducing both systolic and diastolic blood pressure and had a different effect from the negative control. From the test results, it was found that the negative control did not have the same effect as the African leaf extract group, which means that the African leaf extract had an effect on lowering blood pressure compared to the negative control. This is because the negative control was not given any healing treatment.

The results of further tests on the length of time of administration of African leaf extract showed that the length of time of administration had a different effect on blood pressure where the longer the treatment time of African leaf extract the blood pressure decreased, both systolic blood pressure and diastolic blood pressure. The results of the further test of the effect of the most effective African leaf extract were found in the dose group III 200 mg/200 gBW with the highest percentage reduction value of 22.83% with the required administration time of 14 days. The percentage results can be seen in Fig. 1.

The decrease in blood pressure occurs because the African leaf extract contains secondary metabolites in the form of flavonoids. In research conducted by [14] stated that the content of secondary metabolites in the form of flavonoids can reduce blood pressure, this is also related to [15] that flavonoid compounds also have an effect as anticholesterol where cholesterol is one of the factors that can increase blood pressure. According to [16] if cholesterol levels exceed normal limits, atherosclerosis can occur, which if the arterial muscle cells are deposited with fat, it will inhibit the

regulation of blood pressure so that hypertension can occur. The types of flavonoids contained in African leaves are flavonols and flavones [17]. Flavonols and flavones contained in a plant can lower blood pressure by working as an ACE inhibitor [18].

Angiotensin Converting Enzyme (ACE) is a central component of the renin-angiotensin system, which functions to regulate arterial blood pressure and electrolyte balance through the renin-angiotensin-aldosterone system. ACE - Inhibitors are a group of first-line antihypertensives used in several cases of hypertension. The mechanism of action of ACE - Inhibitors is to inhibit the action of the angiotensin converting enzyme which will convert angiotensin I to angiotensin II so that there is no increase in blood volume which can cause blood pressure to increase. Captopril is one of the antihypertensive group that belongs to the ACE-Inhibitor group. The ability of flavonoids as ACE-Inhibitors has been proven in various studies to effectively suppress the work of ACE [18].

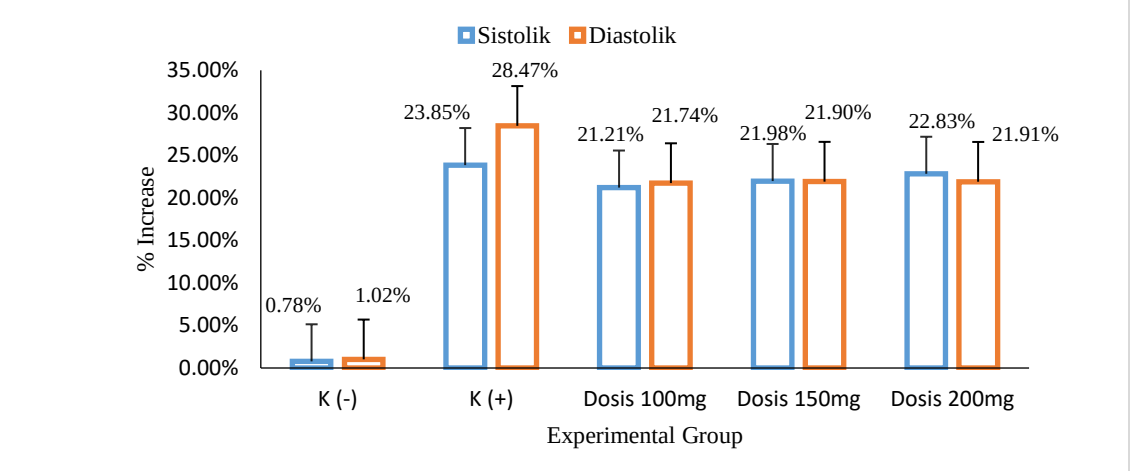

FIGURE 1. Graph of Percentage Drop in Blood Pressure

CONCLUSION

African leaf extract has an antihypertensive effect on male white rats of the Sprague-Dawley strain. The most effective dose of African leaf extract as an antihypertensive in male white rats of the Sprague-Dawley strain was the 200 mg dose group with the highest percentage reduction value of 22.83% with the required administration time of 14 days.

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