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Indonesia has high biodiversity, and some of them has been used for traditional medicine. The villagers of Javanese community in Ponorogo East Java use several plants for hypertension therapy, such as bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*). However, the mechanism of the plants as anti-hypertension is still limited. The pathophysiology of hypertension can be stimulated by oxidative stress that can lead damage to various tissues and implicate in cardiovascular disease. Phenols have a great impact as antioxidants in protecting the cardiovascular system. This research aims to examine the total phenols of extract of bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), dan semangka (*Citrullus lanatus*). The fruits were dried and extracted using methanol absolut, then dried with rotary evaporator. The content of total phenols of extracts were analyzed by using a spectrophotometric method in neutral aqueous solutions react with diazotized sulfanilic acid. The total phenols on bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*) were 363.27 ± 1.44, 335.71 ± 1.44, 599.49 ± 3.61, 409.69 ± 2.16; and 441.33 ± 3.61 respectively.This study suggested that the five plants contain phenols and kumis kucing (*Orthosiphon aristatus*) contents the highest of total phenols.

Keywords:
Total Phenols Content, Antihipertensive Medicinal Plants, Javanese community, East Java Indonesia

1. Introduction

Indonesia is a tropical country with high potential in providing medicinal plants. About 30,000 species of plants from the 40,000 species in the world are in Indonesia, 9,600 of which have been proven to have medicinal properties, and about 300 of them have been used as traditoinal medicines [1]. Some medicinal plants include hypertension therapy. There are sources stating that there are several compounds contained in plants that can play a dual role, namely as angiotensin converting enzyme inhibitors (one type of antihypertensive drug) and as antioxidants [2, 3]. Hypertension is caused by many factors, one of which is the presence of *oxydative stress* [4]. *Oxydative stress* is a chronic imbalance between the antioxidant ability of biological systems and the production of *reactive oxygen species* (ROS). Several experimental and clinical studies indicate that hypertension occurs after the biological system of the body is exposed to *oxydative stress* and an increase in production $\cdot O_2^-$ and H_2O_2 are found in *salt-sensitive and angiotensin II-induced hypertension* cases [5].

Antioxidants are compounds that are important in maintaining a healthy body. These antioxidant compounds work break the chain reaction of free radicals found in the body [6]. The activity of these antioxidant compounds comes from compounds of flavonoids, terpenoids, alkaloids, proanthocyanidin, hydrolyzed, tannins, fatty acids, peptides, xanthones [2] , and phenols [3] . The use of antioxidant compounds is now increasingly

widespread due to the increasing public knowledge of antioxidant functions that can inhibit degenerative diseases such as heart disease,hypertension, and arterisclerosis, cancer and symptoms of aging [6].

Bayam duri (*Amaranthus spinosus*), Temu Hitam (*Curcuma aeru-ginosa*), Kumis Kucing (*Orthosiphon aristatus*), Cincau Hitam (*Mesona chi-nensis*), dan Semangka (*Citrullus lanatus*) are plants that are widely used in Indonesian society, especially Ponorogo as antihypertensive herbs.

From the background above, researchers are interested in comparing phenol levels in both traditional herbal plants which are often used by people around East Java as antihypertensive therapy.

2. Methods & Material

Plant Material

Bayam duri (*Amaranthus spinosus*), Temu Hitam (*Curcuma aeru-ginosa*), Kumis Kucing (*Orthosiphon aristatus*), Cincau Hitam (*Mesona chi-nensis*), dan Semangka (*Citrullus lanatus*) were obtained from the Materia Media Center and around Batu, East Java. Plant determination was carried out at Batu Materia Medica Center.

Plant Extraction

The extraction process requires 1 liter of Absolute Methanol and 100 grams of plant simplicia powder. The method used for extraction is Maseration, where Maseration is done by soaking the simplicia powder in the liquid of the dancer. Sample extraction is done in the Materia Medica Center Laboratory. Each sample is macerated for 24 hours at room temperature avoiding sunlight, then repeating or re-macerating with the same solvent so as to maximize the extraction of the compound's compounds. The extract is then concentrated at temperature 68^{o}C uses a rotary evaporator to get residues consisting of *crude extract* . All extracts are stored in a closed container at 4^{o}C until used.

Qualitative Analysis of Phenolate Compounds

Each extract (0.5 g) was dissolved in chloroform and distilled water (1: 1). The mixture is shaken in a test tube and left for a moment until it forms two layers. The water layer above is used for qualitative examination of phenol compounds. A layer of water is inserted into the drop plate and $AlCl_3$ reagent is added . Positive reaction is indicated by the formation of blue-purple color.

Total Phenolics Content

The tools used in measuring phenol levels are analytic scales, sonicators, centrifuges, closed glass bottles, spectrophotometers, micropipettes. The steps in measuring total phenol levels are: sample solution is added with 1 mL of reagent A (mixture of 7.64% sulfuric acid, H2SO4, 4.8% NaNO2, with a ratio of 5: 1: 5), plus 0.5 mL of reagent B (8% NaOH). The mixture between sample and reagent solution was incubated at 100 ^{o} C for 30-40 minutes, then absorbance was measured by UV-Vis spectropometer at a wavelength of 360 nm. The sample measurement results are compared with phenol as a standard solution. Standard solutions are made using phenol solution with a concentration of 1, 2, 3, 4 ppm with the same treatment, then absorbance is measured.

3. Result and Discussion

Collection of Test Plants

Tabel 1. List of 5 Test Plants

No	Name of Test Plants	Plant Parts Used	Plant Weight (Kg)
1	Bayam duri (*Amaranthus spinosus*)	Leaf	5
2	Temu Hitam (*Curcuma aeruginosa*)	Bulb	5
3	Kumis Kucing (*Orthosiphon aristatus*)	Leaf	5
4	Cincau Hitam (*Mesona chinensis*)	Leaf	5
5	Semangka (*Citrullus lanatus*)	Flesh of Fruit	10

Picture 1. Materia Medica Center (Sample Place Colletion)

The sample collection was carried out starting on May 25-28 2018 at the Materia Medica enter, Batu, Malang, East Java. The materials that will be used in the test are listed in table 1.

Drying of Samples and Simplisia Powder Making

Five samples that have been collected, then cut into smaller pieces to facilitate the drying process. The drying process is carried out for 3-10 days to eliminate the water content in the plant. This makes it easy to make plants into powder. Drying takes place by placing the plant in the sun, so that it takes longer than using a dryer. The drying process is carried out at Materia Medica Center, Batu, Malang, East Java.

Picture 2. Drying Process

After the plants are dried, a simplicia powder is made using a grinding machine. This pollination is intended to expand the contact surface of the simplicia with the mixture so that the process of extracting the compound can be optimal, because the extraction will run more optimally if the powder surface that comes into contact with the liquid of the dancer becomes wider, that is, with relatively small powder. However, too thin a reduction can cause the cell wall to rupture so that solutes are not removed.


Picture 3. Pollination and Powder Results One Simplisia (Temu Hitam)

Sample Extraction

Extraction of medicinal plants using maceration method, a method of extraction with the principle of diffusion of osmosis. The maceration method is used because this method is simple compared to other extraction methods. Maseration is done by soaking the simplicia powder in the liquid of the dancer. Soaking will help infiltrate (penetrate) the liquid of the dancer and soften the cell so that it is easily absorbed. The liquid dancer will penetrate the cell

wall of the plant powder and enter the cell cavity containing the active substance. Withdrawal of the active substance due to differences in concentration between the solution of the active substance in the cell and those outside the cell where the concentration inside is more concentrated than outside the cell so that the liquid with the same polarity as the dancer will dissolve in the penyari then move towards the outside of the cell have a lower concentration. The event occurs repeatedly so that there is a balance of concentration between the solution outside and inside the cell. Maseration is done at room temperature to prevent the loss of volatile substances due to heating. During the maceration process stirring is carried out. This serves to optimize the wetting of the powder so that the whole powder is completely submerged in the dancer. Stirring can also function to prevent the balance of concentration inside and outside the cell so that the diffusion process can continue. The flatter all the submerged parts will be the better because the dancer can enter into all parts of the powder so that the withdrawal of active substances in each cell can be optimized. Then the vessel is closed so that the solvent does not evaporate and mechanical contaminants cannot enter.

After 24 hours, then the maserat is separated by the pulp with the fig filtered and placed in a separate place. Then remaseration is done to get optimal results. After obtaining the mass then concentrating / making thick extracts using a rotary evaporator.

Extraction is done using methanol. The ratio between powder and solvent is 1: 10, where 100 g of simplicia is dissolved in 1 liter of solvent methanol. Making this thick extract uses absolute methanol solvents because it has several advantages over water, which does not cause cell swelling, inhibits enzyme action, and improves the stability of dissolved drug ingredients. Methanol solvents can be used to detect substances with relatively high

polarity until relatively low because methanol is a universal solvent.

Picture 3. Extraction Sample Process

After obtaining the maceration results then concentrating / making thick extracts is carried out. Making thick extracts using a rotary evaporator.

Picture 4. Thick Extract Process

Qualitative Analysis of Phenolate Compounds

Analysis was carried out to determine the qualitative compounds of the presence of phenolic compounds in a sample of Semangka, Kumis Kucing, Bayam Duri, Temu Hitam dan Cincau Hitam. In the 5 samples of methanol extract, the positive results showed that there were phenolic compounds. It can be assumed that the phenolic phenolics are soluble in polar and non-polar solvents. After the qualitative test is carried out, it will be followed by a quantitative test.

Table 2. List of Rendemen 5 Test Plants

No	Plant Name	Simplicia Weight (Gram)	Extration Weight (Gram)	% Rendement
1	Semangka	100	9,82	9,82
2	Kumis Kucing	100	8,39	8,39
3	Bayam Duri	100	8,52	8,52
4	Temu Hitam	100	9,67	9,67
5	Cincau Hitam	100	6,58	6,58

Table 4. Quantitative Tests With Spectrophotometric Methods

No	Plant Name	Parameter	Reagent	Analysis Result	Level of analysis results (mg/L)
1	Semangka	Fenol	Asam Sulfinat	Spektrofotometri	441,33 ± 3,61
2	Kumis Kucing	Fenol	Asam Sulfinat	Spektrofotometri	599,49 ± 3,61
3	Bayam Duri	Fenol	Asam Sulfinat	Spektrofotometri	363,27 ± 1,44
4	Temu Hitam	Fenol	Asam Sulfinat	Spektrofotometri	335,71 ± 1,44
5	Cincau Hitam	Fenol	Asam Sulfinat	Spektrofotometri	409,69 ± 2,16

Quantitative Total Phenol Analysis

Determination of total phenol content in Semangka, Kumis Kucing, Bayam Duri, Temu Hitam dan Cincau Hitam methanol extract were carried out by fig dissolving the sample and then adding 1 mL of reagent A (mixture of sulfuric acid 7.64%, H2SO4, NaNO2 4.8%, with a ratio of 5: 1: 5), plus 0, 5 mL of reagent B (8% NaOH). The mixture between sample and reagent solution was incubated at 100 ^{0} C for 30-40 minutes, then absorbance was measured by UV-Vis spectropometer at a wavelength of 360 nm. The sample measurement results are compared with phenol as a standard solution. Standard solutions are made using phenol solution with a concentration of 1, 2, 3, 4 ppm with the same treatment, then absorbance is measured. From the results of the measurements The total phenols on bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*) were 363.27 ± 1.44, 335.71 ± 1.44, 599.49 ± 3.61, 409.69 ± 2.16; and 441.33 ± 3.61 respectively. This study suggested that the five plants contain phenols and kumis kucing (*Orthosiphon aristatus*) contents the highest of total phenols.

Phenolic compounds are thought to contribute to antioxidant activity in this plant, but have different levels of each extract [7]. This is due to the influence of the plant's growing place, which is supported by the climate and soil elements, causing the total phenolics contained in different plants.

4. Conclusion

From the results of the measurements The total phenols on bayam duri

(*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*) were 363.27 ± 1.44, 335.71 ± 1.44, 599.49 ± 3.61, 409.69 ± 2.16; and 441.33 ± 3.61 respectively. This study suggested that the five plants contain phenols and kumis kucing (*Orthosiphon aristatus*) contents the highest of total phenols.

5. References

1. DEPKESRI, 2007. Keputusan Menteri Kesehatan Republik Indonesia Nomor : 381/Menkes/SK/III/2007 Tanggal 27 Maret 2007. Kebijakan Obat Tradisional Nasional Tahun 2007.
2. Loizzo, M.R. et al., 2007. Inhibition of Angiotensin Converting Enzyme (ACE) by Flavonoids isolated from Ailanthus excelsa (Roxb) (Simaroubaceae). *Phytotherapy Research*, 21(August 2006), pp.32–36.
3. Nugroho, A.E., Malik, A. & Pramono, S., 2013. Total phenolic and flavonoid contents, and in vitro antihypertension activity of purified extract of Indonesian cashew leaves (Anacardium occidentale L .). *International Food Research Journal*, 20(1), pp.299–305.
4. Rodrigo, R., González, J. & Paoletto, F., 2011. The role of oxidative stress in the pathophysiology of hypertension. *Hypertension research : official journal of the Japanese Society of Hypertension*, 34(January), pp.431–440.
5. Larsen, M.K. & Matchkov, V. V, 2016. Hypertension and physical exercise : The role of oxidative stress. *Medicina*, 52(1), pp.19–27. Available at: http://dx.doi.org/10.1016/j.medici.2016.01.005.
6. Halliwell, B. et al., 1995. The characterization of antioxidants. *Food and Chemical Toxicology*, 33(7), pp.601–617.
7. Pourmorad, F., Hosseinimehr, S.J. & Shahabimajd, N., 2006. Antioxidant activity , phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotchnology*, 5(June), pp.1142–1145.

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