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PHYTOCHEMICAL SCREENING, INHIBITION OF ANGIOTENSIN CONVERTING ENZYME ACTIVITY AND ANTI OXIDANT OF EXTRACT PANDAN WANGI LEAVES (*PANDANUS AMARYLLIFOLIUS* ROXB) IN VITRO

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ABSTRACT

Pandan wangi leaves *(Pandanus amaryllifolius* Roxb.) generally only used by the Indonesians people as food additive. Empirically pandan wangi leaves has properties to reduce high blood pressure, and also have the antioxidant activity. To prove this statement a reseach was conducted by blocking the action of Angiotensin Converting Enzyme (ACE) the 70% ethanol extract pandan wangi leaves, using the method of Cushman and Cheung. Antioxidant activity with DPPH Methods. Phytochemical screening also conducted on the powder and 70% ethanol extract pandan wangi leaves and showed secondary metabolites i.e. flavonoids saponins, steroids / triterpenoids and coumarin. The activity results of the ACE inhibitor towards captopril, 70% ethanol extract of pandan wangi leaves, IC_{50} value of 10.17 ± 0.16 bpj; 28.55 ± 1.03 bpj respectively. Antioxidant activity was 70.678 bpj. The conclusion *Pandanus amaryllifolius* Roxb extract have antioxidant activity and also has the activity for inhibition of Angiotensin Converting Enzyme.

KEYWORD: Pandan Wangi Leaf, *Angiotensin Converting Enzyme* (ACE), DPPH.

INTRODUCTION

Hypertension is one of the risk factors for cardiovascular disease that do not rarely cause specific symptoms, with a fairly high prevalence in Indonesia. The prevalence of

hypertension in Indonesia obtained through measurement at ≥18 years of age is 25.8%, the highest in Bangka Belitung 30.9%, followed by South Kalimantan 30.8%, East Kalimantan 29.6% and West Java 29.4%. The prevalence of hypertension in Indonesia was obtained through a questionnaire diagnosed by health workers by 9.4%, who were diagnosed with health workers or were taking medication at 9.5%.[1]

In ancient times authentic Indonesian foods used natural additives from plants to cook which were used as scents, distinctive flavors and color providers. At this time there are still many Indonesians who still use natural ingredients from plants such as cloves, cinnamon, bay leaves, turmeric, orange leaves, ginger, lemongrass, pandan wangi leaves and suji leaves.

One of the plants that is usually used for food additives is pandan wangi leaves (*Pandanus amaryllifolius* Roxb.) Including many Pandanaceae families planted in the yard or gardening. Pandan wangi is generally a green dye and aroma. The use of pandan wangi leaves is not only for food additives but empirically efficacious as antihypertensive. Indonesian people believe that pandan wangi leaves (*P. amaryllifolius* Roxb.) Can be used as antihypertensive drugs. In the previous study, flavonoid compounds from pandan wangi leaves were thought to be efficacious as antidiabetic, antioxidant and antianafilaxis.[2] While in this study testing was conducted to inhibit the work of Angiotensin Converting Enzyme (ACE).In addition to being useful as antidiabetic and antianafilaxis, flavonoid compounds are also beneficial for blood circulation throughout the body, preventing blockages in blood vessels, reducing cholesterol content and reducing fat accumulation in blood vessel walls and reducing the risk of coronary heart disease.[3]

In determining the activity of an herbal, screening method is needed in the form of an in vitro test that is testing using an enzyme that has been isolated, testing the effect of ACE inhibitors by using enzymes isolated from the lungs of rabbits. In vitro testing of ACE inhibition that is often used is the Chusman and Cheung methods. ACE inhibitory activity is known by calculating the percentage decrease in hypuric acid levels formed from the binding of ACE to N-Hippuryl-His-Leu (HHL). Measurement of hypuric acid levels was carried out by Uv-Vis spectrophotometer at a wavelength of 228 nm.[4]

MATERIALS AND METHOD

Matherials

Fresh Pandan wangi leaves (*Pandanus amaryllifolius* Roxb.) Were obtained from BALITRO, Bogor, then made into simplicia Pandan wangi leaves by drying, then mashed. *Angiotensin-Converting* (ACE), Hipuril-L-Histidin-L-Leusin (HHL).

Extract Preparation

Weighed ± 700 grams of simplicity powder, Pandan leaves were put into the maserator, added ± 7 liters of 70% ethanol. Soaked for the first 6 hours while stirring occasionally, then let stand for 18 hours. Maserat is separated by precipitation. The process is repeated 6 times. All maserates were collected, then evaporated with a vacuum evaporator.[5]

Phytochemical Screening

Phytochemical screening included

Ientification of Alkaloids, Identification of Flavonoid groups, Identification of Saponin groups, Identification of Tanin groups, Identification of quinone groups, Identification of steroid and triterpenoid groups, Identification of essential oil groups, Identification of coumarin groups.[6]

Antioxidant Activity.[7]

100, 200, 300, 400 and 500 µL of leaf pandan extract 1000 ppm solution was added to 1 mL methanol solution of DPPH radical (0,04 mM), and then methanol was added until the volume was 5 mL. The mixture was shaken vigorously for 1 minute by vortexing and was left to stand in the dark for 30 minutes at 37°C. Thereafter, the absorbance for the sample was measured using spectrophotometer at 517 nm against methanol blank.

ACE Inhibition activity.[4]

A group of 50.0 µL samples and 50.0 µL substrate solution was pipetted into a test tube, pre-incubated at 37ºC incubator for 15 minutes. A total of 50.0 µL of ACE solution was added to the test tube. Incubation for 30 minutes at 37ºC, 200.0 µL of 1 M HCl were added. Extracted with 1.5 mL ethylacetate, centrifuged at 4000 RPM for 15 minutes. A total of 1.0 mL of the supernatant was pipetted into a test tube and applied to room temperature for 2 hours with the aid of a dryer. After drying, dissolve with 3.0 mL of distilled water and then measured the absorption with an ultraviolet-visible light spectrophotometer at 228 nm.

RESULT AND DISCUSSION

Tabel I: Phytochemical test.

Golongan senyawa	simplicia powder Pandanus wangi leaves wangi	Ekstrak etano l70% Extract of Pandan wangi
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tanin - Galat - Katekuat	 - -	 - -
Kuinon	-	-
Steroi/Triterpenoid	++	++
Minyak Atsiri	-	-
Kumarin	+	+

Note: + = positive reaction

\- = negative reaction

Phytochemical Screening

Plant foods are not only rich in micronutrients but also contain broad variety of biologically active secondary metabolites. These secondary metabolites are responsible for color, flavor, and sometimes, natural toxic to pest or even human. Such substances classification is still under research and debate. Nevertheless, some of them have been referred as protective factors, phytoprotectants, phytochemicals and nutraceuticals.[8]

According to Nix[9], the word phytochemical comes from Greek word phyton, meaning "plant", indicating its chemical nature. As a natural compound, phytochemical serves as either antioxidants or hormones in the originating plant or person eating it. Researchers believe that fruits and vegetables have more than 25,000 phytochemicals, in which many of them have not been identified. Phytochemicals can bring such beneficial effects as a result from synergistic actions of multiple nutrients. Diets high in phytochemicals improve a protective lipid profile to protect against coronary heart disease, improve overall colon function, prevent age-related macular degeneration and cancer, also increase antioxidant status.

Phytochemical screening test was carried out on simplicia powder and pandan wangi leaf extract. The purpose of phytochemical screening is to find out the secondary metabolites found in pandan wangi leaves. The results of phytochemical screening of powder and ethanol

extract 70% of pandan wangi leaves showed secondary metabolites i.e. flavonoids saponins, steroids / triterpenoids and coumarin.

Antioxidant Activity

This research will use DPPH method in order to measure total antioxidant activity of the samples. According to Prakash et al.[10], DPPH method is a rapid, simple, and inexpensive method that widely used to test the ability of compounds as free radical scavengers or electron donors and also to evaluate antioxidant activity. The basic principle of DPPH assay is based on reduction of stable free radical of DPPH by antioxidants. Odd electron in DPPH free radical shows strong maximum absorption at 517 nm and gives purple color. As the molar absorptivity of DPPH radical at 517 nm reduces from 9660 to 1640, the color turns from purple to yellow. This happens when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. Resulting decolorization is stochiometric with respect to number of electrons captured.

Antioxidant test was carried out of pandan wangi leaf extract ethanol 70%, the results of IC_{50} was 70.678 ppm. Results of antioxidant activity measurement can be expressed in terms of IC_{50}. IC_{50} express concentration in ppm samples required for a 50% decrease in absorbance of the DPPH radical. To calculate IC_{50}, a plot of absorbance vs. concentration should be made first (Martirosyan, 2008), the plot can be seen at figure 1.

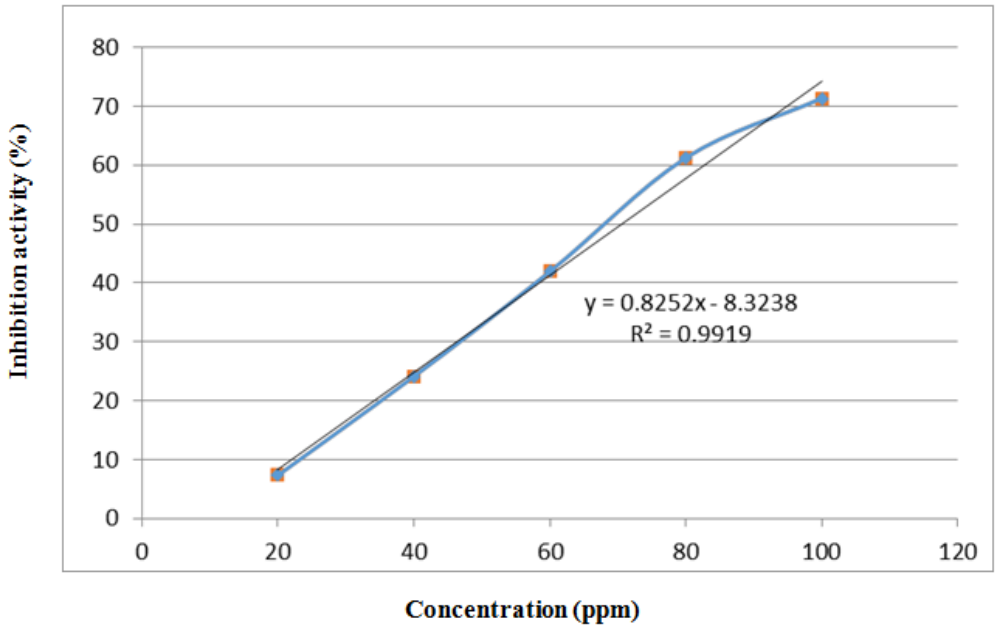

Figure 1: Antioxidant activity of pandan wangi leaf extract.

ACE Inhibition Activity

In this study testing of Angiotensin Converting Enzyme (ACE) inhibitors against captopril, ethanol extract 70% pandan wangi leaves. Whereas captopril is a positive control while extract as a test material. The test material used based on the results of phytochemical screening shows that it contains flavonoid compounds. Flavonoids are secondary metabolites which function to inhibit ACE so that they have an effect as hypotension.[11] Kaptopril and extracts in this study can inhibit the action of ACE as seen from the concentration of hypuric acid and the percentage of inhibition. Hypuric acid itself is produced from the breakdown between ACE and Hipuril-L-Histidine-L-Leusin which is a parameter in this method, the Cushman and Cheung methods[4]

The production of hypurat acid standard curve aims to obtain a linear regression equation. On the hypurat acid standard curve, the linear regression equation is obtained, namely y = 0.0569x + 0.0162. The equation will be used to convert the absorption obtained from captopril and ethanol extract 70% pandan wangi leaves so that the concentration of hypuric acid and percentage of inhibition will be obtained. The percentage of inhibition was and the correlation curve was made between the percentage of inhibition (y axis) and concentration (x axis) of captopril and ethanol extract 70% of pandan wangi leaves so that the obtained curves would get the linear equation used to calculate IC_{50} values. The relationship curve between percentage inhibition and concentration of captopril can be seen in figure 2, for ethanol extract 70% pandan wangi leaves can be seen in figure 3.

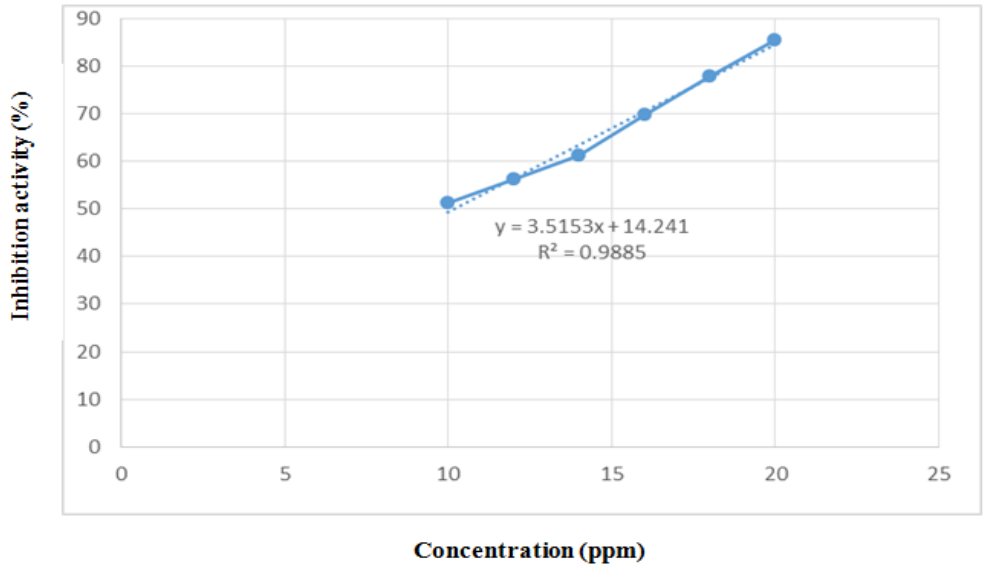

Figure 2. ACE inhibition activity of captopril.

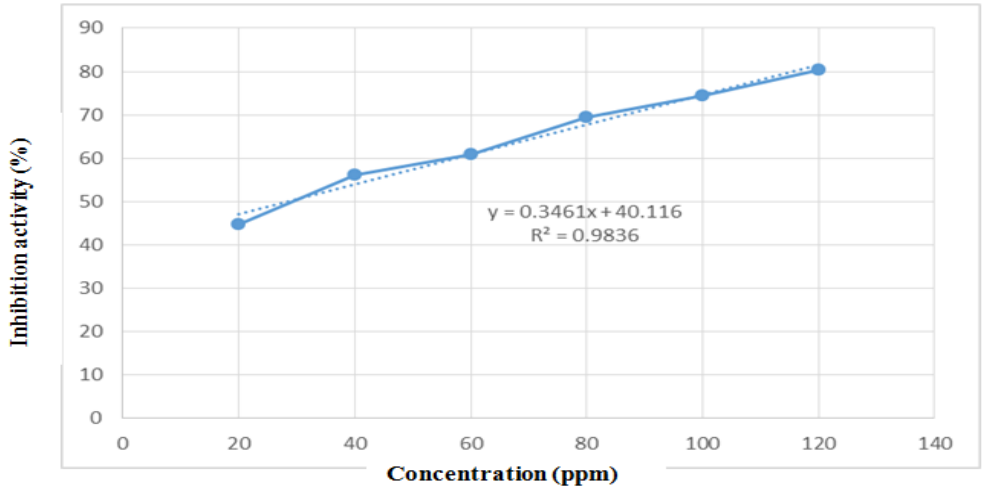

Figure 3. ACE inhibition activity of pandan wangi leaf extract.

IC_{50} values were calculated to determine the amount of captopril concentration and extracts which could inhibit 50% of ACE work. From the data obtained, showed that captopril used as a positive control had IC_{50} values of 10.17 ± 0.16 ppm. Based on the IC_{50} captopril value from a previous study entitled The Effect of Inhibition of ACE Activities of Some Medicinal Plants in Indonesia, which amounted to 13.69 BPJ, it can be said that the method used was appropriate because the IC_{50} values obtained in this study were not significantly different from the literature already available. For the test, ethanol extract 70% pandan wangi leaves obtained IC_{50} value of 28.55 ± 1.03 ppm. When viewed based on the results above, the IC_{50} value of captopril showed smaller than ethanol extract 70% pandan wangi leaves.

CONCLUSION

The conclusion of this study is *Pandanus amaryllifolius* Roxb extract have antioxidant activity and also has the activity for inhibition of Angiotensin Converting Enzyme.

Pandanus amaryllifolius Roxb leaf extract has secondary metabolit contents flavonoids saponins, steroids / triterpenoids and coumarin.

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