IN VITRO INHIBITION OF WATER EXTRACT OF KUMIS KUCING AND TEMPUYUNG TOWARDS ANGIOTENSIN CONVERTING ENZYME ACTIVITY

[1]DYAH ISWANTINI, [2]MIN RAHMINIWATI, [3]HAYYIN NUR ROHSELA, [4]LATIFAH K DARUSMAN

[1,2,4]Biopharmaca Research Center, Bogor Agricultural University, Bogor, Jl. Taman Kencana no 3 Bogor, Indonesia.
[1,3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Gedung Kimia, Jl Agathis, IPB Darmaga, Bogor, Indonesia.
E-mail: [1]dyahprado@gmail.com

Abstract - Angiotensin Converting Enzyme (ACE) has an important role in increasing blood pressure (hypertension). ACE works with the mechanism of the conversion inactive decapeptide angiotensin I to angiotensin II as its active form. With the formation of angiotensin II, there will be a narrowing of the blood vessels which can lead to hypertension. *Kumis kucing* (*Orthosiphon aristatus* (Blume) Miq.) and *tempuyung* (*Sonchus arvensis*) plant were extracted through maceration method using water and yield respectively 10.7% and 8.63%. Based on shrimp larvae toxicity test with BSLT methods, Extract of *kumis kucing* and *tempuyung* had LC_{50} values at 1789.06 and 1657.44 ppm. ACE inhibitory activity were tested with ACE Kit-WST method and obtained respectively 69.20% for single extract of *kumis kucing*, 39.67% for single extract of *tempuyung*, and 88.34% for captopryl at concentration 25 ppm. Based on the results, a single extract of *kumis kucing* have a higher power inhibition than tempuyung but lower than captopryl as a positive control. While the combination of the two extracts did not yield a good inhibition power. The inhibition power of *kumis kucing's* single extract at a concentration of 50 ppm is high enough to be a natural ACE inhibitors and may potentially as an antihypertensive.

Keywords - *kumis kucing* (*Orthosiphon aristatus* (Blume) Miq.), *tempuyung* (*Sonchus arvensis*), Angiotensin Converting Enzyme (ACE), inhibition, *in vitro.*

I. INTRODUCTION

Indonesian people have long known recognizing and using medical herbs as an effort to maintain the health treatment. Until now its traditional usage is still observed as the alternative medicine along with the modern medicine. It indicates a strong tendency for utilizing natural products to keep human health. *Kumis kucing* (*Orthosiphon aristatus* (Blume) Miq.) and field sowthistle (*Sonchus arvensis*) are known as medicinal herbs. [1] stated that there is an antihipertensive compound which was isolated well from *kumis kucing*'s leaf. The ethanol extract of both sample which are shown as an antihipertensive herbal medicine with the mechanism of ACE inhibition had been conducted [2]. According to that research, it was known that 50 ppm of *kumis kucing*'s extract and 14 ppm of field sowthistle's extract had an inhibition potency up to 76.98% and 62.89% respectively. *Angiotensin Converting Enzyme* (*ACE*, peptidyl-dipeptide hydrolase EC 3.4.15.1) is a metaloenzyme containing Zinc as the cofactor. It can be found in vein's endothellium tissue of lungs and regulating an important role of blood pressure [3]. ACE becomes one of the factor for blood pressure increasement, by converting inactive decaptide of Angiotensin I to Angiotensin II as an active speciment [4]. This conversion by ACE which is an exopeptidase is happened by releasing dipeptide of C-terminal from Angiotensin I to form Angiotensin II as a very hypertensive compound [5]. As Angiotensin II formed, blood vessel constriction happens and leads to hypertension. Consumption of antihypertensive medicine bring side effects like hypersensitivity symptom, such as itchy rash, and respiratory tract infection symptom, like cough [4]. Consequently, a further research and development for inventing a safer ACE inhibitor, innovative, and economically reasonable in order to prevent or treat hypertension is needed.

Commonly, a bioactive compound which has function as ACE inhibitor is groupped as flavonoids. Several plants except *kumis kucing* dan field sowthistle, which have been inspected, contains flavonoid and have function as an antihypertensive, such as *Hibiscus Sabdariffa* [5], and *Apple peel extract* [6]. One of flavonoid compund which can be a support to their activity as ACE inhibitor is quercetin. Quercetin is known to decrease blood pressure by inhibiting ACE enzyme [7]. An active quercetin compound becomes one of the flavonoids which has been inspected as antihypertensive by *in vitro* [8] And this research is aimed to evaluate the inhibiting potency of *kumis kucing* leaf and field sowthistle to ACE activity by *in vitro* as the potency of an antihypertensive.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures Materials

Kumis kucing and *tempuyung* were collected from Bogor (Central Java, Indonesia). The Tween-80, quersetin, and *ACE Kit-WST* were purchased from Sigma chemical Co., USA.

Procedures

2.2 Preparation of Plant Sample Extraction

Sample extraction used dried sample as much as ±5 g with water as solvent in a volume of 500 ml (3×24 jam) by maceration method then the mixture was filtered. The obtained filtrate then was concentrated with rotavapor until the concentrated extract was reulted, then dried by vacuum drier and stored in -20 °C until analysis. In the further steps water extract will be writen as *extract*.

2.3. Determination of total flavonoid content [9].

Extract was weighed equal to 200 mg of simplicia then put inside of round boiling flask. A hydrolysis system was performed, and filtered again using cotton. Ethyl acetate fraction was collected inside of a 50 mL volumetric flask, and the remained volume was filled by ethyl acetate. That mixture then was taken. The remained solution later was filled by glassial acetic acid 5% (v/v). Absorbance was measured by using spectrophotometer in wavelength of 425 nm with quercetin as the standard.

2.4 Determination of Inhibition Performance to ACE Activity .

Inhibition activity of ACE was tested by using *ACE inhibitory assay kit (ACE kit-WST, Dojindo Laboratories, Kumamoto, Japan).* Absorbance measurement was performed by using *microplate reader* in the wavelength of 450 nm. Inhibition activity of ACE by the sample then was calculated according to the formula writen on the manual procedure.

III. RESULTS AND DISCUSSION

Kumis kucing and field sowthistle extracts were obtained from *Pusat Studi Biofarmaka*, Bogor. Those extracts were produced after a maceration process for 3×24 hours. Water was choosen as the solvent based on the previous research conducted by Iswantini *et al.* (2011) [2]. As of that, the yield extract could be in a big amount. The yields obtained in this research were 10.7% and 8.63% for *kumis kucing* and field sowthistle extracts respectively. Those extracts later were examined its metabolic secondary contents, the toxicity level against shrimp larvae, inhibition performance against ACE activity by *in vitro*, and total flavonoid compunds contents.

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3.1 Total Flavonoid Contents

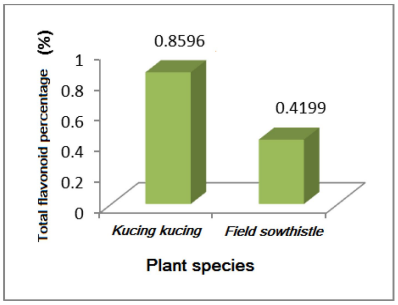

Fig. 1. Total flavonoid contents of both tested extract.

By determining the total flavonoid contents, indirectly quercetin amount inside the extract can be easily measured. It is because quercetin is commonly found as the active compound in the herbal medicine and has pharmacologic activity. In this project, the total flavonoid compound was performed using colorimetric method with $AlCl_3$ as the chromogenic reagent.

The result shows both of the extracts have total flavonoid contents below 1% (**Fig.1**). Hence, those flavonoids of both sample are considered as a minor constituent.

3.2. Toxicity Test against Shrimp Larvae

By and large, a natural material used for medicinal purpose needs to be measured its toxicity level. LC_{50} *(Letha1 Concentration$_{50}$)* is an extract concentration which may cause 50% of the mortality to the shrimp larvae after 24 hours of incubation. Both of the extract were passed by utilizing BSLT (*Brine Shrimp Lethality Test*) method on 48 hours of Artemia salina larvae. The number of dead larvae is shown in Attachment 2 and LC_{50} value by using Probit Quant is shown on **Fig. 2**.

According to Fig. 2, it is shown that both extract act as non-toxic because both extract have value of LC_{50} above 1000 ppm, 1789.06 and 1657.44 ppm for *kumis kucing* and field sowthisle extract respectively. Those valuses indicate a low bioactivity possesed by both plants. On the other side, it is expected that both extract will not a toxic effect for long term of continuous consumption to human body. If any extract has high bioactivity potency, it is not a guarantee to have a biggest inhibition power because LC_{50} is used as the border for medicine formulation.

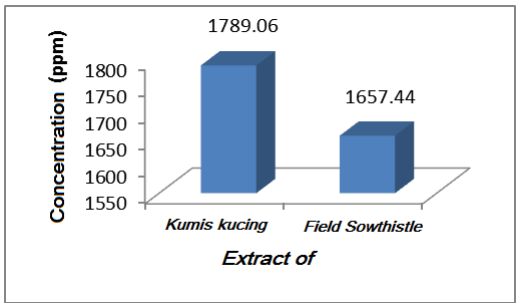

Fig. 2. LC_{50} value of both plant extracts against *A. salina* larvae

3.3 Extract Inhibition Potency against ACE Activity by In Vitro

In this research, both extract were examined for its inhibitive property against ACE by *in vitro* in a various concentration of 50, 100 and 150 ppm. These concentrations were below LC_{50}. This concentration accordance aimed to determine the inhibitive activity and supposed to be safe for human, so no toxic symptoms occurred. Captopril was used as the positive control because it is known for high level of inhibition against ACE activity and used frequently by society as antihypertensive medicine. By

comparing it to the captopril, we would have a rough comparison between the both extracts and captopril, whether it can be as an alternative medicine against hypertension.

The result of the inhibition test of each single extract, mixture extract, and captopril was written in Attachement 3 and graphically shown in Fig. 3.

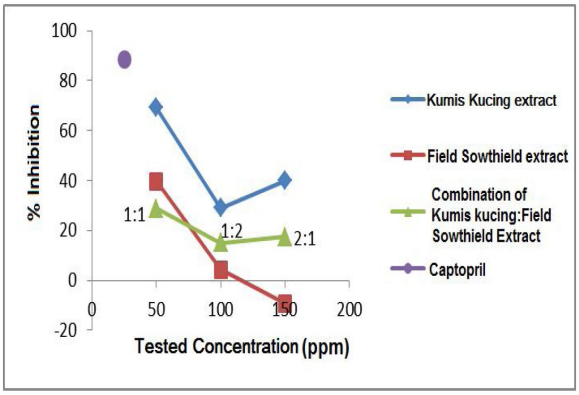

Fig. 3. Inhibition test of the samples by *in vitro* against ACE activity.

The result also shown that the inhibition potency of *kumis kucing* extract (69.20%) was bigger than the field sowthistle extract (39.67%) at the same concentration level (50 ppm). Many previous reports had shown that a plant which has rich phytochemical contents had an effective inhibition of ACE activity [10]. Besides, Iswantini *et al.* (2011) [2] also stated that a richer amount of secondary metabolite would influence to its inhibitive property. A combination of both extract was hypothesized would increase the inhibition percentage for the better result of medicine formulation in a large scale medicinal industry. However, the result did not given any better result compared to the single extract and captropril significantly. Captopril as the positive control still gave the highest inhibition value.

According to the result, a single content of *kumis kucing* has a considerable alternative inhibitive property against ACE at 50 ppm. But, an extract combination from both plant will not bring any better result. A further research for varied and higher level of *kumis kucing* concentration is needed to be discovered. Hence a better and comprehensive understanding will be gained, whether at a certain level kumis kucing extract has the highest inhibitive property, otherwise it activating ACE activity.

CONCLUSIONS

Single extract of *kumis kucing* 50 ppm has the largest inhibition performance up to 69.20% compared to field sowthistle (39.67%) in the same concentration. Combining the extract gave no better result for inhibition. Based on the result, kumis kucing extract is able to be a natural inhibitor of ACE, and has potency as the antihypertensive medicine at the concentration of 50 ppm.

ACKNOWLEDGMENTS

Author would like to thanks to Indonesian Government who provided funding for this reasearch.

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