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 L.C50 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 antihypertensive compound which was isolated well from *kumis kucing*’s leaf. The ethanol extract of both sample which are shown as an antihypertensive 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 endothelium 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 decapetide of Angiotensin I to Angiotensin II as an active specimen [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 grouped 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 compound 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, quercetin, 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 resulted, then dried by vacuum drier and stored in -20 °C until analysis. In the further steps water extract will be written 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 written 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 chosen 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 extract 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 compounds contents.

3.1 Total Flavonoid Contents

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 AlCl3 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. LC50 (Lethal 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 LC50 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 LC50 above 1000 ppm, 1789.06 and 1657.44 ppm for kumis kucing and field sowthistle extract respectively. Those values indicate a low bioactivity possessed 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 LC50 is used as the border for medicine formulation.

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 LC50. This concentration accordence 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
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compared 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 Attachment 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 captopril 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.

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REFERENCES


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