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Angiotensin I-converting enzyme inhibitory activity of tartaric acid and optical isomers

Graduate School of Chosun University

Department of Life Science

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ABBREVIATION

ACE	Angiotensin I converting enzyme
ACEi	Angiotensin I converting enzyme inhibitor
LPS	Lipopolysaccharide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
HHL	Hippuryl-his-leu
HA	Hippuric acid
HPLC	High pressure liquid chromatography
RAS	Renin-angiotensin system
Ang I	Angiotensin I
Ang II	Angiotensin II

ABSTRACT

주석산과 그 이성질체의 안지오텐신 전환 효소 억제 효과

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고혈압은 심혈관계 질환의 위험요소로 전세계적으로 성인의 15-20%가 앓고 있다. 하지만 고혈압의 정확한 발생원인은 밝혀지지 않았다. 다만, 한 가지 요소가 아닌 다양한 요소에 의해 고혈압이 발생한다고 하는 의견에 힘이 실리고 있다. 이 다양한 요소 중 하나인 Renin-angiotensin system (RAS)는 혈압조절에 중요한 역할을 한다. 안지오텐신 I 이 안지오텐신 전환 효소 억제제 (ACE)에 의해 안지오텐신 II 로 변화 되고 이 안지오텐신 II 는 안지오텐신 II 수용체에 결합하여 나트륨 이온의 재흡수와 칼륨 이온의 배출을 증가시키고 혈류량을 늘리는 알도스테론을 생성하게 되고 결과적으로 혈압이 상승하게 된다.
안지오텐신 전환 효소 억제제는 안지오텐신 I 이 안지오텐신 II 로 변화되는 것을 억제하고 지연시켜 결과적으로 혈관수축을 억제하게 된다. 이러한 효능으로

Captopril (D-3-mer-capto-2methylpropanoyl-L-proline)과 같은 안지오텐신 전환 효소 억제제는 항고혈압 약제로 널리 사용되고 있다.

본 연구는 앞서 발표한 솔잎에서 추출해낸 항고혈압물질의 구조를 기반으로 하여 32개의 화합물의 안지오텐신 전환 효소의 억제효과를 측정하였고, 그 중 효과가 뛰어난 주석산의 이성질체에 따른 억제효과에 관한것이다.

주석산은 2개의 히드록시기가 있는 디카르복시산으로 Malic acid와 함께 포도에 자연적으로 들어 있는 산이다. 포도주를 만들 때 침전하는 주석에 함유되어 있어 주석산이라 불리기도 하며 시럽, 주스 등에 널리 이용되며 염색공업, 제과 유기합성, 금속의 착색 등에 사용된다. 주로 천연에 존재하는 L-주석산과 D-주석산이 D-주석산과 L-주석산의의 동량의 혼합체인 Racemic DL-주석산은 포도산이라 불리며 광학적으로 분리해내면 천연에 존재하지 않는 D-주석산을 분리 해낼 수 있으며 광학적으로 분리 불가능한 Meso-주석산과 같은 이성질체가 있다.

본 연구는 이러한 주석산과 그 이성질체, 주석산의 이성질체를 동량 혼합 하였을 때의 안지오텐신 전환 효소 (ACE)의 억제 효능과 안지오텐신 전환효소와 주석산 간의 결합을 autodocking을 이용하여 확인한 결과 DL-주석산은 85.56 ± 7.57%, D-주석산은 88.28 ± 4.24%, L-주석산은 91.74 ± 3.23%, Meso-주석산 69.27 ± 20.58%을 나타 내었고, 각각의 이성질체를 동량으로 혼합한 혼합체인, D: L은 87.57 ± 9.44 %, D: M은 93.14 ± 4.25%, L: M 94.35 ± 4.06%의 활성을 나타내었다. 이 결과는 대표적인 안지오텐신 전환 효소 억제제인 Captopril과 유사한 활성이었다.. 또한, 실제로 ACE binding assay를 위하여 *E.coil*에 mouse ACE gene을 construction하여 발현 시켰다. 약제로써의 사용을 위하여 MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)를 이용하여 세포에 대한 독성을 확인하였다.

ABSTRACT

Angiotensin I-converting enzyme inhibitory activity of tartaric acid and optical isomers

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The Angiotensin converting enzyme (ACE) is the key enzyme catalyzes angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile) to angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile) in renin-angiotensin system. Angiotensin I stimulate aldosterone secretion by binding with angiotensin II receptor, then secreted aldosterone regulates contraction and relaxation of a blood vessels through release or absorption of Water and Na+.Inhibitory activities against angiotensin I-converting enzyme (ACE) of tartaric acids and optical isomers were investigated using variety buffer in vitro. Tartaric acid has a variety types; DL-tartaric acid, D-tartaric acid, L-tartaric acid and Meso-tartaric acid form. Using borate buffer, DL-tartaric acid 58.53 ± 15.47%. Using HEPES buffer. DL-tartaric acid, 58.53 ± 15.42%. Using Tris-HCl buffer, DL-tartaric acid 78.49 ± 20.42% and Using Tris-Zinc buffer, DL-tartaric acid; 98.64 ± 3.12% ACE inhibition of DL-tartaric acid was strong and stability ACE inhibition using Tris-zinc buffer. The

subsequent ACE inhibitory activity was using a Tris-zinc buffer. The ACE inhibitory activity assay was performed by measuring the concentration of hippuric acid liberated from HHL by the method described by Cushman and Cheung. DL-Tartaric acid (DL) was 85.56 ± 7.57%, D-Tartaric acid (D) 88.28 ± 4.24%, L-tartaric acid (L) 91.74 ± 3.23% and Meso-Tartaric acid 69.27 ± 20.58%. When in 1:1 ratio Tartaric acids, D-tartaric acid: L-tartaric acid (D: L) 87.57 ± 9.44 %, D-Tartaric acid: Meso-tartaric acid (D: M) 93.14 ± 4.25% and, L-tartaric acid: Meso-tartaric acid (L: M) 94.35 ± 4.06%. The ACE inhibitory activity of tartaric acid was similar to captopril as a commercial ACE inhibitor. And we analyzed its binding stability when been interfered with small molecule to obtain drug candidate for anti-hypertension therapy. Furthermore we identified ACE-tartaric acid complex. Here we found tartaric acid from ZINC database that potentially abrogate ACE-ANG I interaction using Virtual Screening. And Construction for structure study of ACE and inhibitor binding assay. Cytotoxicity of tartaric acids was determined using MTT assay in SVEC 4-10 cell line. Tartaric acid has not cytotoxicity.

INTRODUCTION

Cardiovascular disease (CVD) is responsible for one-third of global deaths and is a leading and increasing contributor to the global disease burden (Diane et al., 2002). Importantly, CVD is eminently preventable. In order to achieve significant reductions in the avoidable CVD burden, a combination of population-based and high-risk strategies necessary. These strategies should target lifestyle-related risk factors such as unhealthy diet, physical inactivity and tobacco use, as well as the intermediated mainfestations of these lifestyles; hypertension, glucose intolerance, and hyperlipidemia. In addition, strategies aimed at improving management of those already affected by CVD should be an integral component of a comprehensive approach for the prevention and control of CVD. (WHO and ISH writing group 2003)

Hypertension is already a highly prevalent risk factor for CVD throughout the industrialized world. It is becoming an increasingly common heath problem worldwide because of increasing longevity and prevalence of contributing factors such as obesity, physical inactivity and an unhealthy diet (Wada et al., 1985; Savluchinsake et al., 1997)). The current prevalence in many developing countries, particularly in urban societies, is already as high as those seen in developed countries (Tolmacheva et al., 2006; Tagat et al., 1994). Hypertension is related to cerebrovascular disease, ischemic heart disease, cardiac and renal failure. Treating hypertension has been associated with about a 40% reduction in the risk of stroke and about a 15% reduction in the risk of myocardial infarction (Kinouchi et al., 2000). Although the treatment of hypertension has been shown to prevent CVD and to extend and enhance life, hypertension remains inadequately managed everywhere (Prinz et al., 2002; Vidotti et al., 2004; Yoon et al., 2000; Bernsterin et al., 1989; Acharya et al., 2003; Ehlers et al., 1989; Unger 2002). In addition, hypertension often coexists with other cardiovascular risk factors, such as tobacco use, diabetes, hyperlipidemia and obesity, which

compound the cardiovascular risk attributable to hypertension (Prinz et al., 2002; Vidotti et al., 2004; Yoon et al., 2000).

The renin angiotensin system (RAS) is important system of blood pressure, homeostasis, electrolyte balance (Kalupahana, 2012), and this system have various enzymatic cascade. Angiotensinogen, the main substrate of RAS, convert to finally angiotensin II through two step. Renin is secreted by specialized epithelioid cells of the kidneys in response to a decrease in intravascular volume or Na+ level (Boon, 1997). Renin cleaves the peptide bond, the leucine and valine, on angiotensinogen to form ten peptide angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), then ACE divide two amino acid His-Leu (C-terminal) of angiotensin I to form eight amino acid peptide angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) (Steven, 2012). Physiological effect of angiotensin II appear through Angiotensin II receptor, type 1 (AT1 receptor) and angiotensin II receptor, type 2 (AT2 receptor). Angiotensin II stimulate AT1 receptor which induces vasoconstriction and secret aldosterone from the adrenal cortex. The vasoconstriction and aldosterone secretion increased blood pressure and sodium and water retention. But Diterpene resin acids are an important compound to prevent herbivores and pathogens (Diane et al., 2002). The biological activity of diterpene resin acids, such as antimicrobial, antiulcer, and cardiovascular activities is the most represented for this class of diterpenoids. Dehydroabietic acid and its derivatives exhibit antiulcer (Wada et al., 1985), antimicrobial (Savluchinske et al., 1997), anxiolytic (Tolmacheva et al., 2006) antiviral (Tagat et al., 1994), antitumor (Kinouchi et al., 2000), and cytotoxic activities (Prinz et al., 2002).

The effect of two chiral carbon atoms in its molecular structure, tartaric acid has four different optical isomers, namely DL-, D-, L- and Meso-tartaric acid. L-tartaric acid is a natural organic acid occurring widely in fruits, especially in grapes and berries. In industrial manufacturing, natural products are still the main source of L-tartaric acid, while only DL-tartaric acid can be produced by chemical synthesis. In recent years, biosynthetic

pathways of tartaric acid have been attracting more and more attention for producing L- or D-tartaric acid of high purity and safety, which are the requirements in food and drug quality and management (Yang, W., et al, 2013).

In a previous study, Park et al. (2015), determined ACE inhibition activity of pinus densiflora by three method. First, The ACE inhibition activity was examined by measuring the concentration of hippuric acid isolated from HHL by the modified method of Cushman and Cheung (Cushman, 1970). second, Expression of Angiotensin II receptor, type 1 and endothelial nitric oxide synthase 3 (eNOS) in mouse endothelial cells. Third, Constructs for structure study of ACE and inhibitor binding assay. This study, ACE inhibition activity was examined by modified method of Park et al. (2015) We found that tartaric acid possess antihypertension effects using angiotensin I-converting enzyme (ACE) inhibition assay by HPLC and the molecular docking suites Autodock and Vina and demonstrate how the combination of docking and visualization can aid structure-based drug design efforts.. We tested ACE inhibitory activities of tartaric acid and optical isomers.

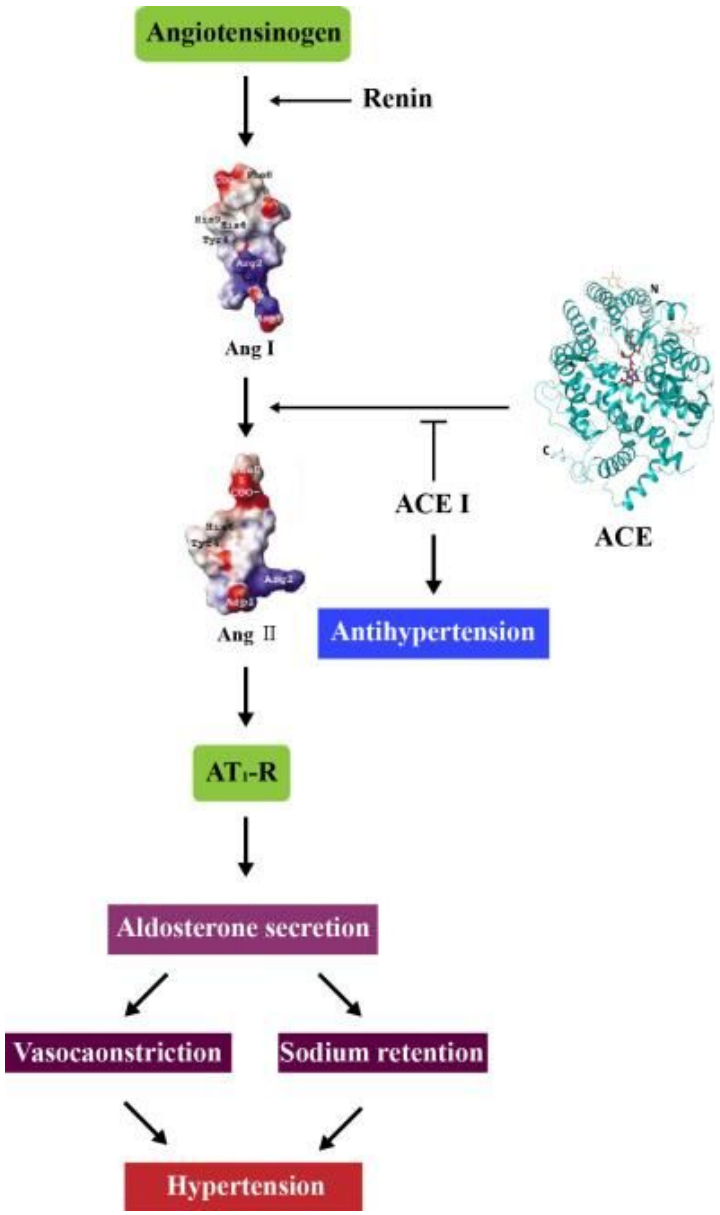

Fig. 1. The classical renin angiotensin converting enzyme pathway. ACE; angiotensin converting enzyme, ACE I; angiotensin converting enzyme inhibitor, Ang Ⅰ; angiotensin Ⅰ, Ang Ⅱ; angiotensin Ⅱ, AT1-R; angiotensin Ⅱ receptor (type Ⅰ)

MATERIALS AND METHODS

1. Reagents

Chemicals and enzymes were obtained as follows: angiotensin-converting enzyme (from rabbit lung), HHL, HA, DL-, D-, L-tartaric acid, and other chemicals. (Sigma, St. Louis, MO, USA); Meso-tartaric acid (Santa cruz, NJ, USA), HPLC-grade acetonitrile (Fisher Scientific, Nepean, Canada). All other chemicals were of reagent grade and also obtained from Fisher Scientific.

2. HPLC

Reversed-phase HPLC with a pack polymer C18 column (6 um particle size, 4.6 mm, 250 mm) from the YMC Corporation, and a Shimadzu HPLC system (Tokyo, Japan) consisting of a LC-20AT pump, CTO-10AS column oven, and an SPD-20A UV detector was employed. The mobile phase was composed of different proportions of flow A : ACN, and flow B : 0.1% phosphoric acid in water (acidified water). The initial mobile phase composition was 12.5% A and 87.5% B, followed by a linear gradient to 12.5% A and 87.5% B in 20 min; 30-45 min, from 87.5% to 100% A. The post-running time was 5 min. The flow rate was 1 mL/min, the column temperature was set at 25 °C, and the sample injection volume was 20 uL.

3. ACE inhibitory activity assay

The ACE inhibitory activity assay was performed by measuring the concentration of hippuric acid liberated from HHL by the method described by Jianping Wu (Wu, J., et al, 2002.) with slight modifications. Hippuryl-his-leu (HHL) hydrolysis were carried out for 30 min at 37 °C. Each mixture contained the following components at the indicated final concentration: 150 uL of buffer (containing 5 mM HHL and one of tartaric acid 3mM) and 100 uL of the enzyme. Buffer used four types; borate buffer (50 mM pH 8.5 containing 300 mM NaCl, Chen, J., et al, 2013), HEPES buffer (50 mM pH 8.5 containing 300 mM NaCl, Pennanen, N., et al, 1995), Tris-HCl buffer (50 mM pH 7.5 containing NaCl 300mM) and Tris-zinc buffer (Tris-HCl 150 mM pH 8.3 containing 1 uM Zn, Parvathy, S., et al, 1997). The ACE was added last to initiate the reaction. An aliquot of 60 uL of sample or distilled water was added to the sample or control mixture, respectively. The enzymatic reactions were terminated by addition 250 uL of 1 N HCl; the HCl was added before the enzyme in zero-time all blank assays. The hippuric acid was extracted from the acidified solution into 1.5 mL of ethyl acetate by vortex mixing for 15 sec. After centrifugation, a 1.0 mL supernatant was transferred to a clean tube. The supernatant were evaporated by heating at 60 °C for 1 h in a vacuum pump. The hippuric acid was dissolved in 1.0 mL of distilled water. To calculate ACE inhibition rate, the hippuric acid was measure at 228 nm. As positive control, captopril (3 mM) was used.

The hippuric acid was dissolved in 1.0 mL of distilled water. To calculate ACE inhibition rate, the hippuric acid was measure at 228 nm. The ACE inhibition rate was calculated as follow:

$$\text{ACE inhibition rate (\%)} = \frac{(A_{228\ \text{Control}} - A_{228\ \text{Test}})}{A_{228\ \text{Control}}} \times 100$$

where A and B are the test samples without and with compound or fraction.

4. Cell culture

The simian virus 40 transfected mouse lymph node endothelial cell line SVEC4-10, was obtained from the American Type Culture Collection (ATCC; Manassas, VA) and cultured following ATCC guidelines. Cells were maintained in DMEM with 4.5 g/L glucose and 10% FBS and 1% antibiotic and culture medium renewed every 48 hours.

5. Cytotoxicity

MTT assay was performed by the method of Mosmann in the modification of Hansen, Nielsen, and Berg as Kuzma, Wysokinska, Rozalski, Krajewska, and Kisiel described previously (Pennanen et al 1995; Boncler et al 2014). Briefly, Raw 264.7 cell in suspension were seeded at 2×10^4 cells per well in 96-well plate and these cells were grown in a humidified atmosphere of 5% CO2 in air at 37 °C. Then the cells were exposed to varying concentrations of the Pine needle extract (200 ug/mL to 25 ug/mL) for 24h. After that, culture medium was added 100 uL fresh one. MTT solution (5 mg/mL PBS) was then added and the plate was located in optimal atmosphere at 37 °C. The metabolically active cells reduced MTT to blue formazan crystals. After 4 h, MTT-formazan crystals were dissolved in 50% ethanol and 50% DMSO and absorbance was measured at 570 nm on a multifunctional plate reader (Eon, Bio-tek, USA) and compared with untreated cells.

6. RNA isolated from mouse and cDNA synthesis

Total RNA was isolated from house mouse tissues using Hybrid-R (GeneAll) according to the manufacturer's protocol. 500 uL of RiboEx reagent was added and incubated to homogenized tissue samples for 5 min at the room temperature. The sample centrifuge at 12,000 × g for 10 min at 4 °C. and transfer the supernatant to a fresh tube. 100 uL of chloroform was added to samples, mixed and store for 2 min at room temperature. The mixture centrifuge at 12,000 × g for 15 min at 4 °C. and transfer the aqueous phase to a fresh tube. 50% ethanol added to the sample and transfer to a mini spin column. The samples centrifuged at 10,000 × g for 30 sec at room temperature. 500 uL RBW was added to the mini spin column and was centrifuged at 10,000 × g for 30 sec at room temperature. Then, 500 uL RNW was added the mini spin column and was centrifuged at 10,000 × g for 30 sec at room temperature. Total RNA was harvested and synthesized cDNA. cDNA was synthesized by using the Superscript First-strand synthesis system, also according to the manufacturer's established protocol (Invitrogen, Carlsbad, CA). Thereafter, it was reverse-transcript as following program: 65 °C. 10 min, 42 °C. 50 min, 70 °C. 15 min, and the cDNA product was stored at - 80 °C.

7. Constructs for structure study of ACE

pET-15b vector including the His·tag and T7 promoter was used for crystal structures of ACE in complex with dehydroabietic acid and communic acid (Fig. 13) (Bernsterin et al., 1989). For generation of expression clones, all inserts were ligated into 5-Nde I and 3-BamH I sites of a pET-15b vector. To prepare insert DNA, ACE form mouse was RT-PCR amplified from cDNA isolated using the GeneAll kit. RT-PCR was performed with n-pfu polymerase (Enzynomics) and the sequence of the PCR primers were: for the sense strand amplification, a forward primer 5'-TAC ATA TGG AAA ACC TGT ATT TTC AGG GCA TGC TCG ACC CTG GAT TGC-3' named ACE-F7-8 and a reverse primer 5'-TAG GAT CCT CAG GAG TGT CTG AGC TCC ACC TCA GAC CCA A-3' named ACE-R7-9. BamH I and Nde I restriction sites were used for cloning the ACE gene. RT-PCR amplification was performed with Hot-start method and products were examined by gel electrophoresis on 1% agarose gels. Cloning The cloned were confirmed by restriction enzymes. The restriction enzyme was used BamH I and Nde I or BamH I and EcoR I.

8. Confirm transformation on DH5α and sequencing

Sequencing reactions are performed in the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) using the ABI BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the protocols supplied by the manufacturer.

Single-pass sequencing is performed on each template using primer; pET-F1 (GGG TTA TGC TAG TTA TTG CTC AG), ACE-F11 (CCT TGG TGA TGC TTC CAT CA), ACE-F12 (AGT ACT CCA GCA GTG CCT T), ACE-F13 (CTA CGC AGA GAG ACG TGC A), ACE-F14 (CAA CTT GGC ATA GCT TCG TG), and ACE-F15 (CAT GGC TTC CGT GGC ATC TA).

Fluorescently labeled fragments Applied Biosystems is purified by a method recommended with removing the unincorporated dNTPs and the terminator. The samples are injected to electrophoresis in an ABI 3730xl DNA Analyzer (Applied Biosystems).

DNA sequences were translated to protein and aligned using the Gene Runner (5.0.59 Beta) and Clustal-Omega programs.

9. Ligand docking and binding site analysis with PyMOL, PyRx and Autodock/Vina

The model of ACE (1O8A) was obtained from PDB BANK (http://www.rscb.org) and structure of captopril (ZINC 00020226) and L-tartaric acid (ZINC 00895301) was downloaded from zincdatabase (http://zinc.docking.org), then its energy form were minimized and converted to pdbqt format by Open Babel in PyRx 0.8 as ligand for virtual screening. Identifications molecules targeted ACE was done by docking of the Captopril and L-tartaric acid from ZINC database against ACE-binding domain of Captopril and L-tartaric acid using AutoDock Vina in PyRx 0.8 (Virtual Screening Tools) (Trott O et. al 2010). Virtual screening is now established as an effective paradigm for filtering compounds for drug discovery process (Jalaie, M., et al. 2006).. The grid for docking calculations was centered on ACE-binding domain of Captopril and L-tartaric acid. The best three of drug-like compounds from virtual screening were taken on the basis of higher scoring function for pharmachopore modeling, which is one of the most powerful techniques to classify and identify key features from a group of molecules. Ligand Scout was used to develop pharmacophore and molecular interaction of tartaric acid.

RESULTS

ACE inhibitory activity assay

The ACE inhibition activity was examined by measuring the concentration of hippuric acid isolated from HHL by the modified method of Jianping Wu (Wu, J., et al, 2002). The results of the previous research showed that ACE inhibition activity of .dehydroabietic acid and communic acid isolated from pinus densiflora (Park et al, 2015) (Fig. 2). The ACE inhibition activities of dehydroabietic acid and communic acid showed 74.8 % and 69.9%, respectively (Fig. 3). Tested antihypertension effects of 32 chemicals focusing hydroxy group and carboxyl group chemical structure (Fig. 4-7). The 19 chemicals showed ACE inhibitory activity. The benzoic acid 38.51.± 1.21%, 2,3-diaminobenzoic acid 68.79 ± 10.54%, trimesic acid 29.96 ± 5.41%, Poly(acrylic acid) 78.14 ± 2.14%, DL-tartaric acid 96.14 ± 2.53%, 2-fluoro-6-hydroxybenzoic acid 45.20 ± 7.42%, 2-fluroic acid 59.57 ± 6.54%, Glutaconic acid 80.24 ± 3.65%, 1-cyclopentenecarboxylic acid 33.89 ± 1.77%, pipecolinic acid 12.59 ± 6.31%, 2-amino-3,4,5-trimethoxybenzoic acid 45.50 ± 6.48%, 2-amino-5-nitrobenzoic acid 61.92 ± 8.47%, benzeneseleninic acid 76.84 ± 21.57%, cyclohexeane-1,2,4,5-tetracarboxylic acid 80.67 ± 9.40%, 2,5-thiophenedicarboxylic acid 82.48 ± 8.87%, DL-proline 37.67 ± 4.21%, D-proline 68.70 ± 1.27, L-proline 20.44 ± 10.12% and glycolic acid 47.58 ± 3.65%. This resuls showed Fig 9. The tartaric acid showed high and stability ACE inhibitory activity. The ACE inhibition of DL-tartaric acids in various buffers was shown in Figure 10. Using borate buffer, DL-tartaric acid 58.53 ± 15.47%. Using HEPES buffer. DL-tartaric acid, 58.53 ± 15.42%. Using Tris-HCl buffer, DL-tartaric acid 78.49 ± 20.42% and Using Tris-Zinc buffer, DL-tartaric acid; 98.64 ± 3.12% ACE inhibition of DL-tartaric acid was strong and stability ACE inhibition using Tris-zinc buffer. The subsequent ACE inhibitory activity was using a Tris-zinc buffer. DL-Tartaric acid (DL) was 85.56 ± 7.57%, D-Tartaric acid (D) 88.28 ± 4.24%, L-tartaric acid (L) 91.74 ± 3.23% and Meso-Tartaric acid 69.27 ± 20.58%. When mix in 1:1 ratio Tartaric acids, D-tartaric acid: L-tartaric acid (D: L) 87.57 ± 9.44 %, D-Tartaric acid:

Meso-tartaric acid (D: M) 93.14 ± 4.25% and, L-tartaric acid: Meso-tartaric acid (L: M) 94.35 ± 4.06%. Particularly in L: M-tartaric acid, higher ACE inhibitory activity than other tartaric acid. This result was shown in Figure 11.

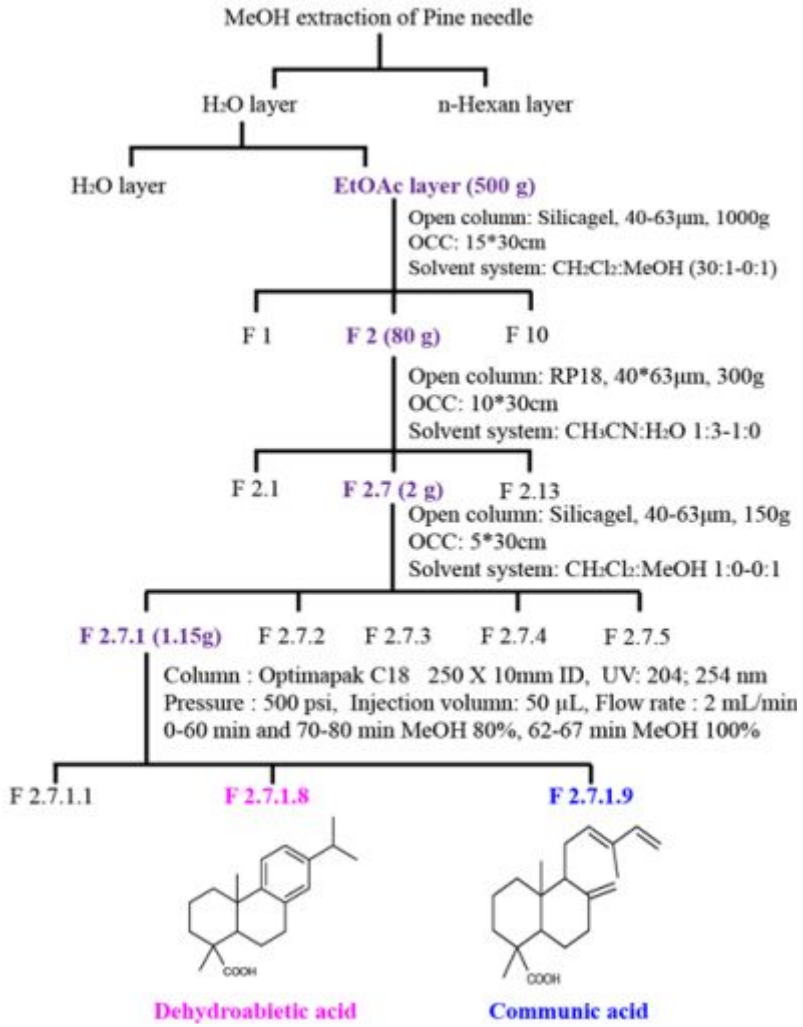

Fig. 2. Procedure for isolating dehydroabietic acid and communic acid from pine needle. The ethyl acetate-soluble fraction exhibiting strong fibrinolysis activity was further purified by repeated silica gel column and HPLC, and the n-butyl alcohol-soluble fraction was purified with a Sephadex LH-20 column and HPLC. The structures of dehydroabietic acid and shikimic acid from the ethyl acetate-soluble fraction was identified by 1H NMR and 13C NMR spectroscopy. (Park et al, 2015)

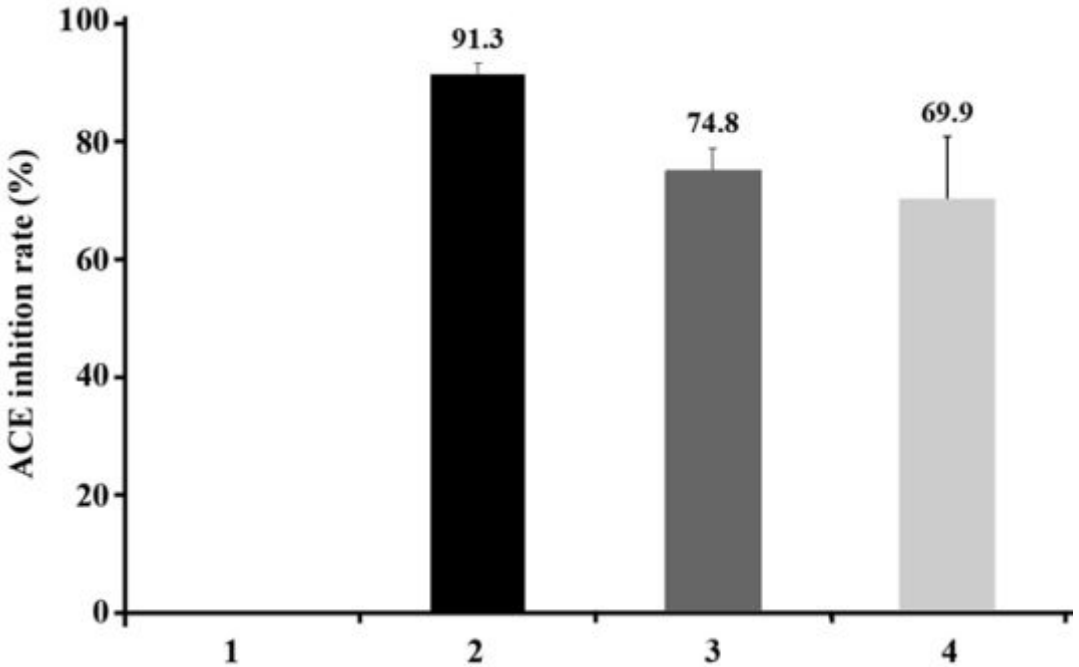

Fig. 3. Angiotensin converting enzyme inhibitory effect of isolated compound from ethyl acetate fractions. 1: Negative control, 2: 3 mM captopril, 3: 3 mM dehydroabietic acid, 4: 3 mM communic acid. Bars represent means ± SD (n=3). (Park et al, 2015)

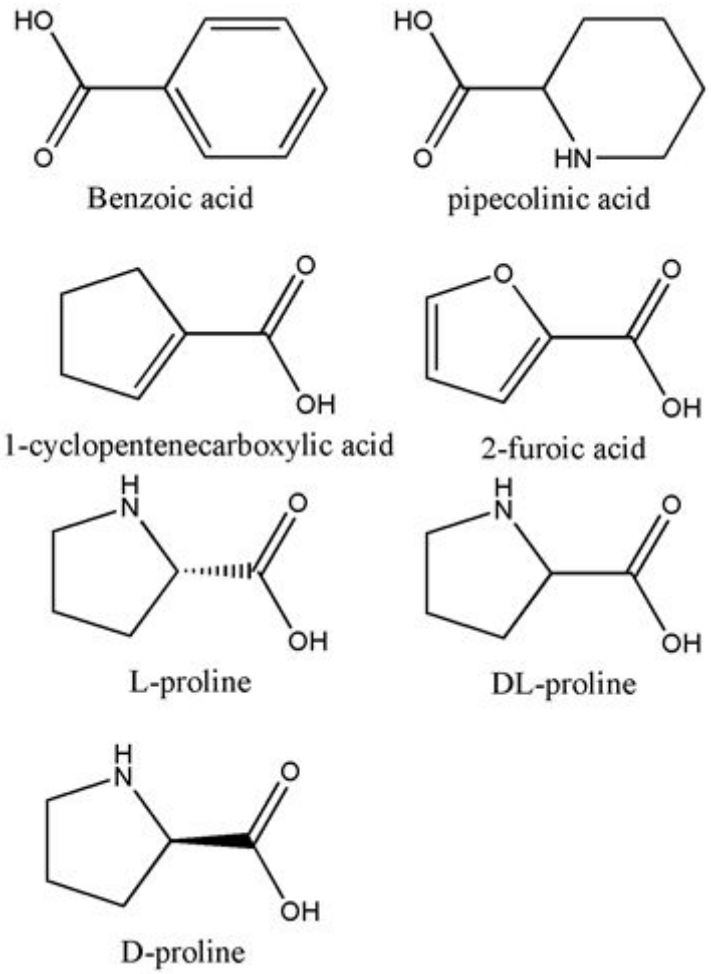

Fig. 4. Chemicals having carboxyl group and other .residues

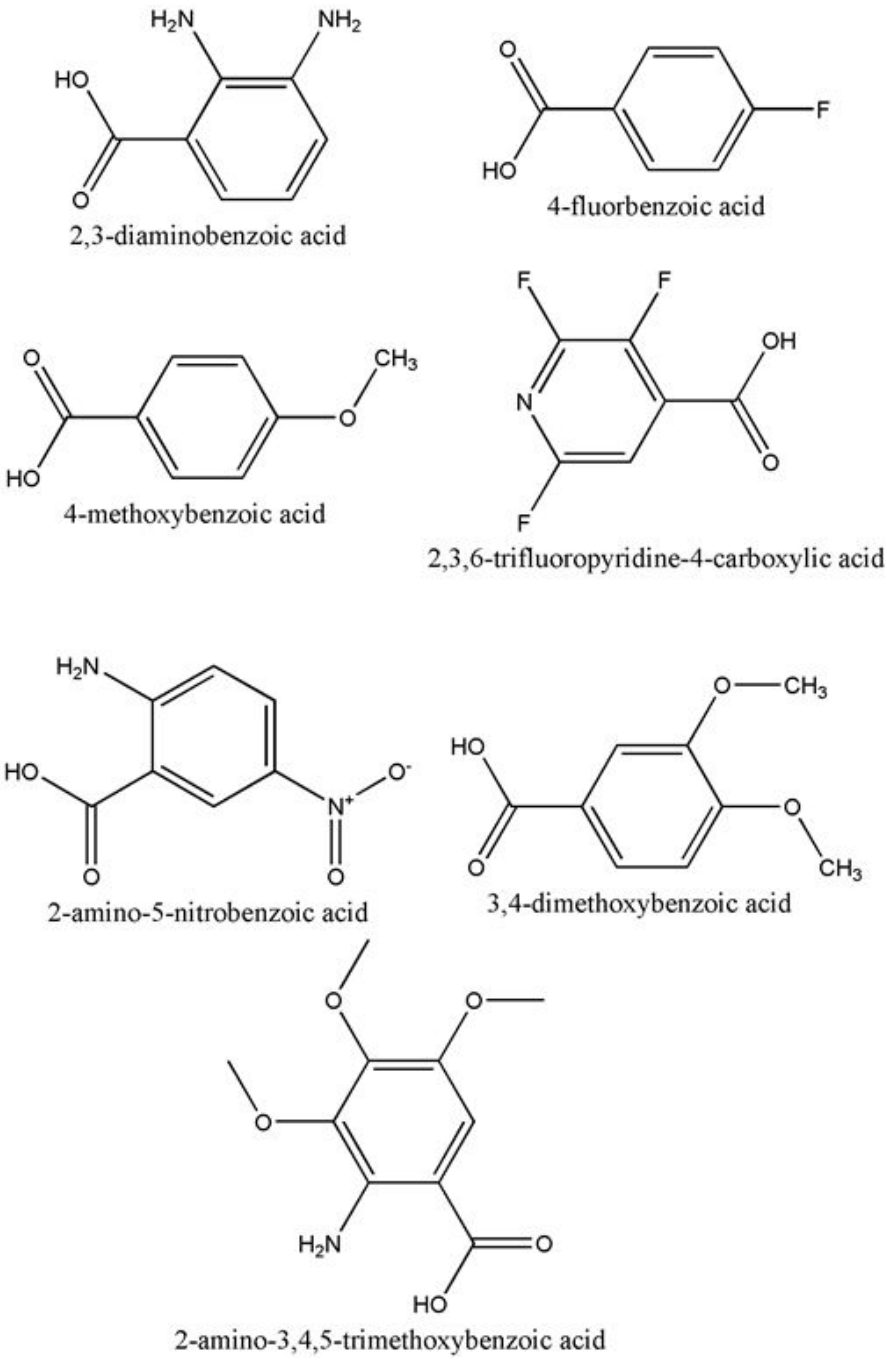

Fig. 5. Chemicals having only carboxyl group and other .residues

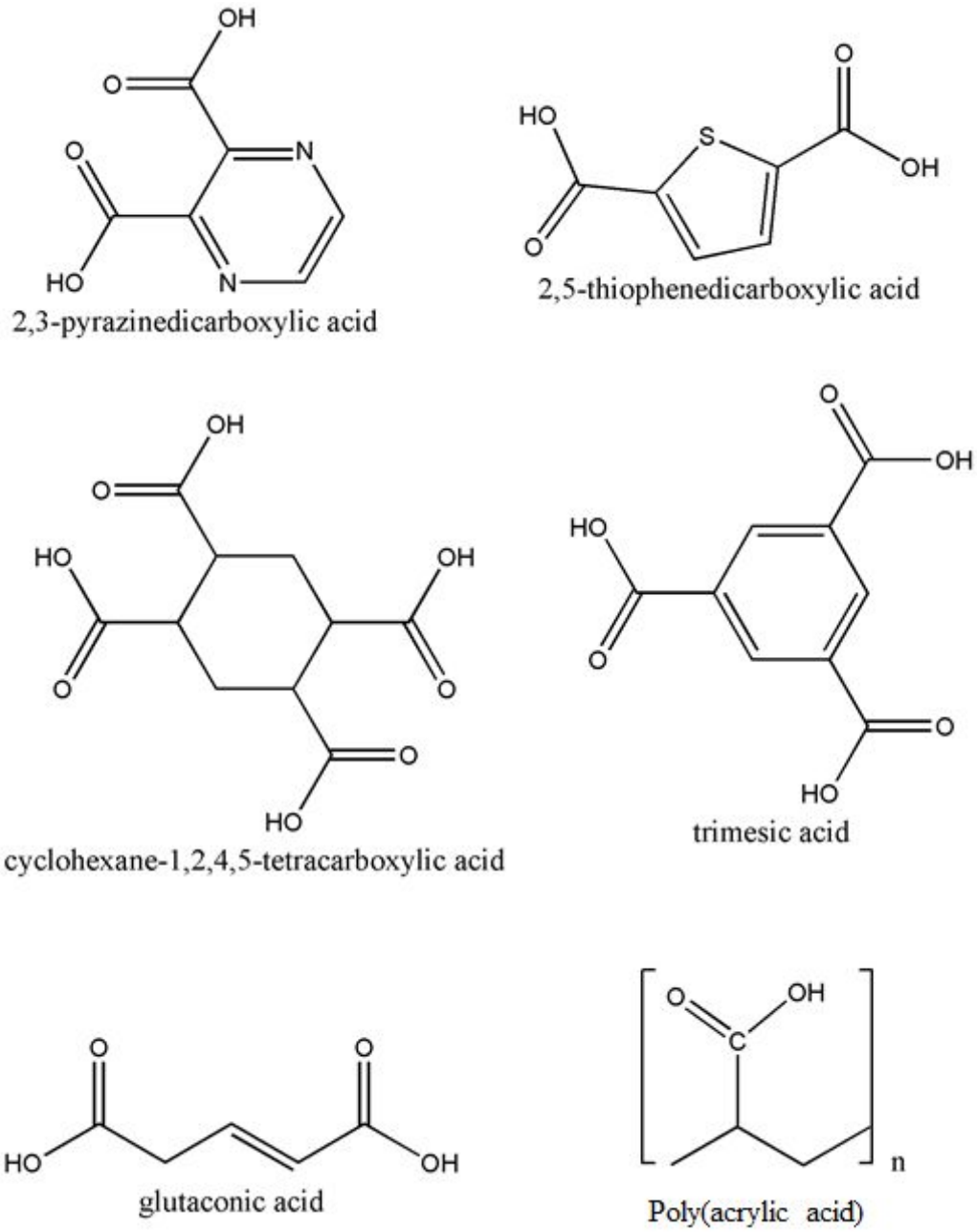

Fig. 6. Chemicals having only one carboxyl group.

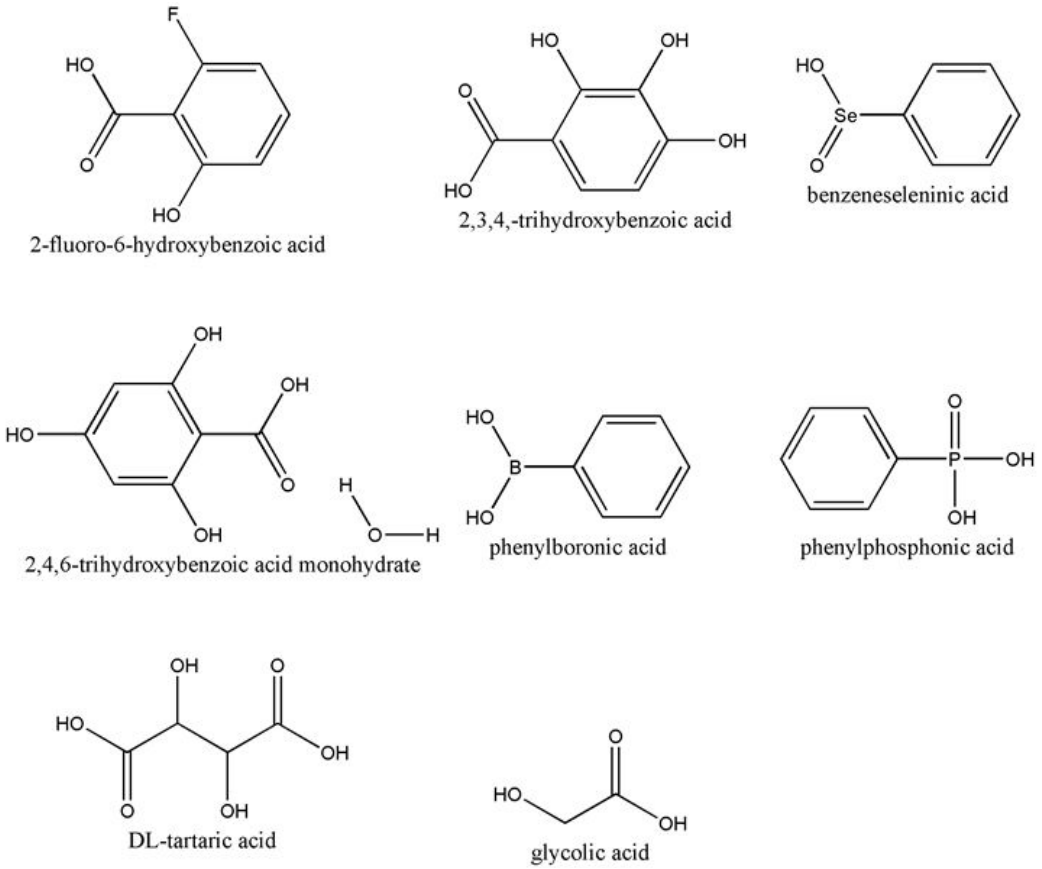

Fig. 7. Chemicals having hydroxy group and other .residues

1

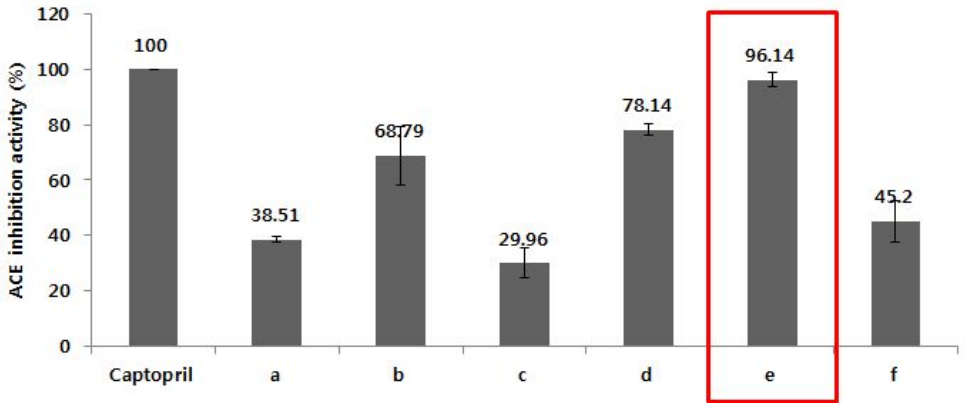

Fig. 8-1. Determined ACE inhibitory activity of chemicals. a; benzoic acid 38.51.± 1.21%, b; 2,3-diaminobenzoic acid 68.79 ± 10.54%, c; trimesic acid 29.96 ± 5.41%, d; Poly(acrylic acid) 78.14 ± 2.14%, e; DL-tartaric acid 96.14 ± 2.53%, f; 2-fluoro-6-hydroxybenzoic acid 45.20 ± 7.42%,

2

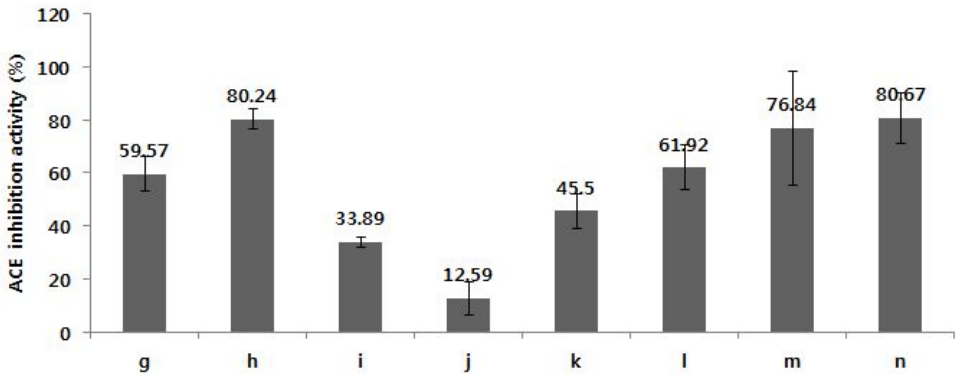

Fig. 8-2. Determined ACE inhibitory activity of chemicals. g; 2-fluroic acid 59.57 ± 6.54%, h; glutaconic acid 80.24 ± 3.65%, i; 1-cyclopentenecarboxylic acid 33.89 ± 1.77%, j; pipecolinic acid 12.59 ± 6.31%, k; 2-amino-3,4,5-trimethoxybenzoic acid 45.50 ± 6.48%, l; 2-amino-5-nitrobenzoic acid 61.92 ± 8.47%, m; benzeneseleninic acid 76.84 ± 21.57

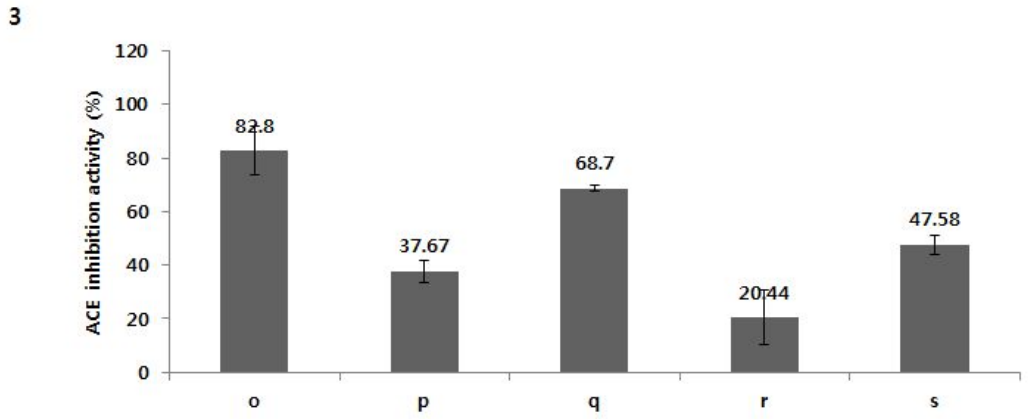

Fig. 8-3. Determined ACE inhibitory activity of chemicals. n; cyclohexeane-1,2,4,5-tetracarboxylic acid 80.67 ± 9.40%, o; 2,5-thiophenedicarboxylic acid 82.48 ± 8.87%, p; DL-proline 37.67 ± 4.21%, q; D-proline 68.70 ± 1.27, r; L-proline 20.44 ± 10.12% and s; glycolic acid 47.58 ± 3.65%.

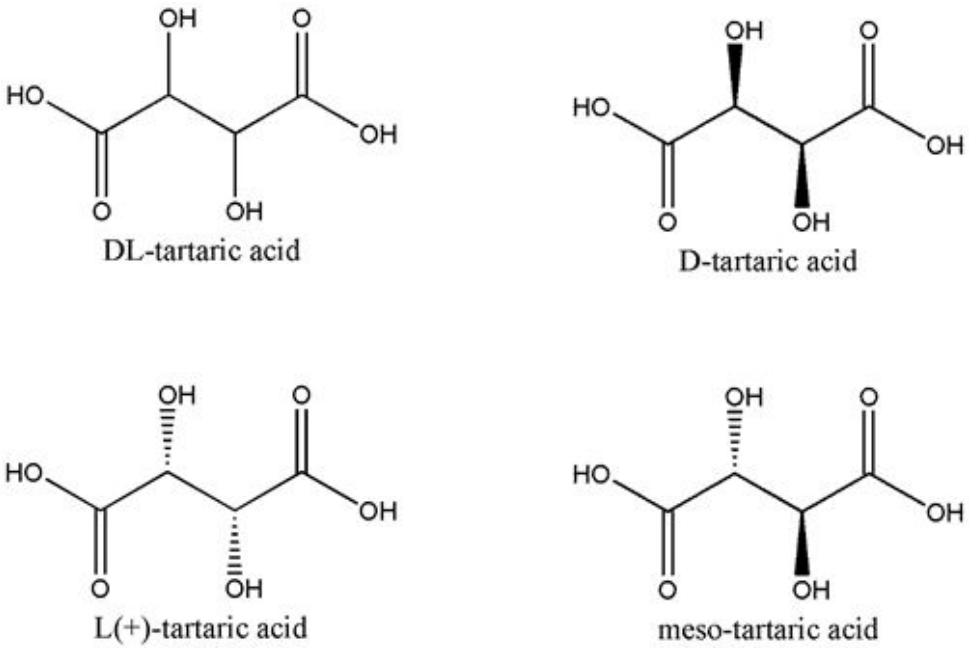

Fig. 9. Structure of tartaric acid and optical isomers

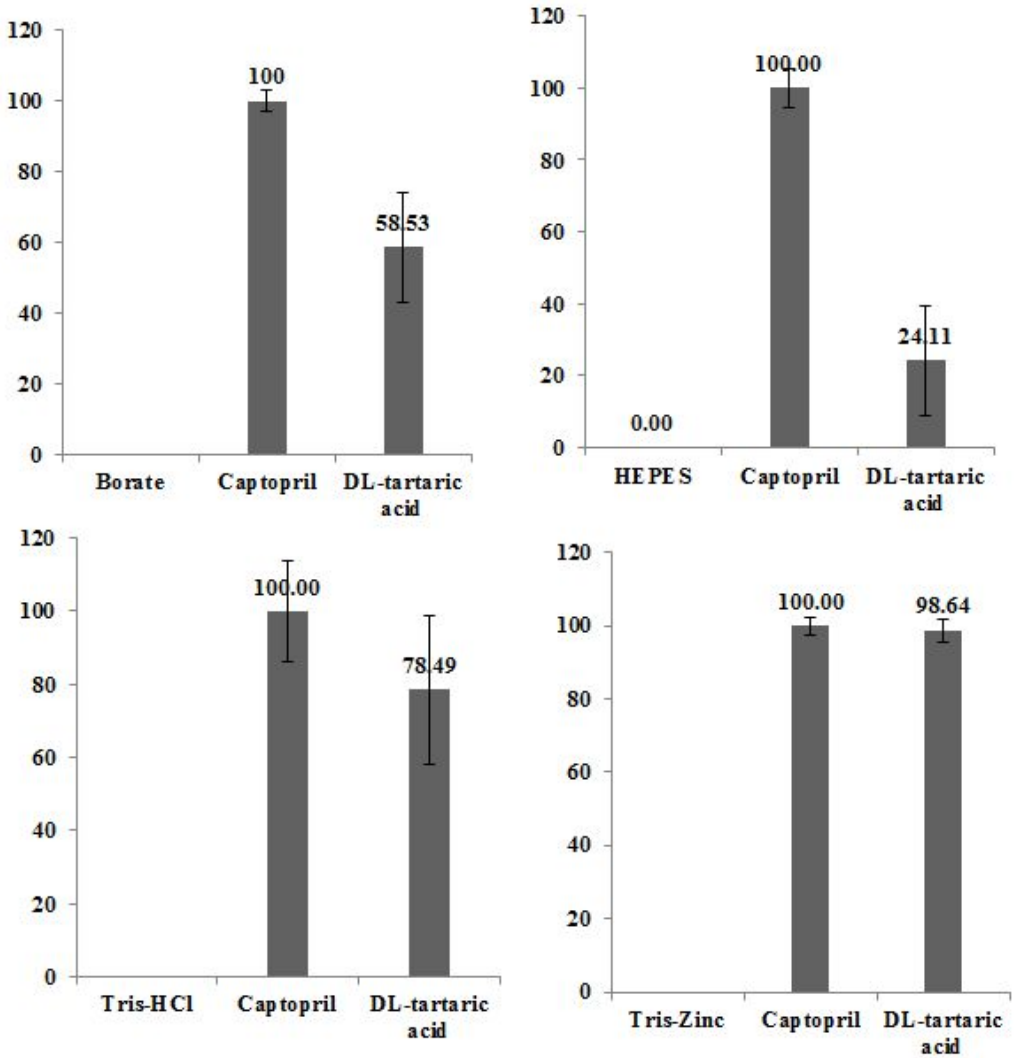

Fig. 10. Determined ACE inhibitory activity of DL-tartaric acid using four buffer. Borate buffer; Captopril ; 100 ± 3.014%, DL-tartaric acid; 58.53 ± 15.47%,
HEPES buffer; Captopril ; 100 ± 5.43%, DL-tartaric acid; 58.53 ± 15.42%,
Tris-HCl buffer; Captopril ; 100 ± 13.95%, DL-tartaric acid; 78.49 ± 20.42%
Tris-Zinc buffer; Captopril ; 100 ± 2.4%, DL-tartaric acid; 98.64 ± 3.12%

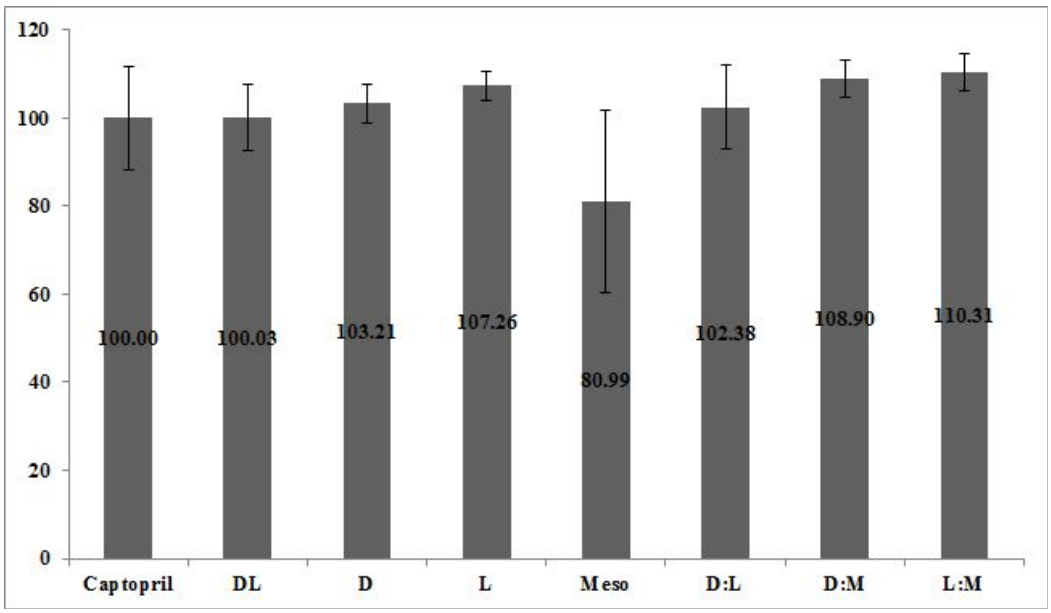

Fig. 11. ACE inhibitory activity of tartaric acid, optical isomers and mixture of isomer. Captopril ; 100 ± 11.80%, DL-; 100.03 ± 7.57%, D-; 103.21 ± 4.24, L-; 107.26 ± 3.233, Meso-; 80.99 ± 20.589, D:L (in 1:1 ratio); 102.38 ± 9.44%, D:M (in 1:1 ratio); 108.90 ± 4.25%, L:M (in 1:1 ratio); 110.31 ± 4.06%.

Cytotoxicity

The cytotoxic effects of seven tartaric acids at molecular and cellular levels were tested on RAW264.7 cultured cell lines. The results indicate that all tartaric acids no cytotoxic effects at concentrations of 25 μg/mL to 100 μg/mL on three tested cultured cell lines, as shown in Figure 12.

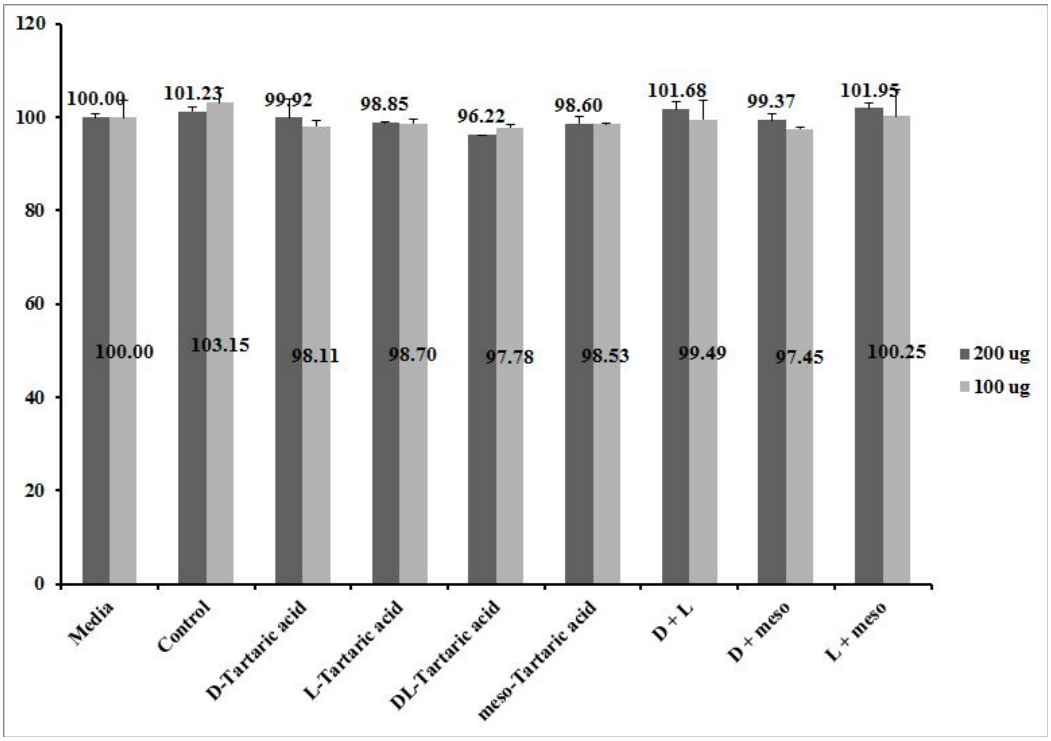

Fig 12. Cell viability of tartaric acid on RAW 264.7 cells. Tartaric acids were applied to the cells for 24h and cell viability was assessed by MTT assay as described in the text. Tartaric acids had effect on cell viability.

Construction of Angiotensin converting enzyme gene

The sequence of house mouse is used NCBI database. Assession number of used ACE sequence is NM-207624.5 and only the mature protein sequence was used. DNA size is 3.831 bp and RT-PCR product is 3,874 bp, including ATG, TAG, BamH Ⅰ (GGATCC), Nde Ⅰ (CATATG), and TEV sequence (5'-GAA AAC CTG TAT TTT CAG GGC-3'). TEV sequence is needed to separate ACE protein (Fig. 14).

To cloning mature ACE gene, RT-PCR was carried out using ACE-F7-8 and ACE-R7-9 or ACE-7-10. 1 and 3 were confirmed that the synthesized with ACE full sequence. 2 and 4 were not used to generate the multi-band (Fig. 15). PCR products and pET-15b vector were ligation used to T4 ligase, and pET-15b-ACE was transformed to DH5α.

Confirm transformation on DH5α and sequencing

To confirm the transformation, it was digested by several restriction enzymes, BamH Ⅰ, EcoR Ⅰ, and Nde Ⅰ. BamH Ⅰ and Nde Ⅰ will cut insert DNA and vector, and the size of band is predicted 5,696 bp and 3,865 bp, respectively. The 18 colony was confirmed, and it was identified that the five colony clipped to the expected size, 918, 919, 926, 929, and 937 (Fig. 16).

Also, BamH Ⅰ and EcoR Ⅰ will cut insert DNA and vector, and the size of band is predicted 6,621 bp and 2,976 bp, respectively. The 18 colony was confirmed, and it was identified that the five colony clipped to the expected size, 918, 919, 926, 929, and 937 (Fig. 17). ACE gene was found that entered in the opposite direction to the pET-15b.

The 918, 919, 926, 929, and 937 colony were confirmed sequence. Sequence alignment used to Clustal Omega (HTTP://www2.ebi.uk/clustal omega/). The mouse ACE (NM 207624.5) and pET15b-ACE 937 proteins share 100% amino acid identity. Figure 19 shows

a sequence alignment of pET15b-ACE 937 and mouse ACE. The pET15b-ACE 918 and 926 were not correspond with mouse ACE (NM 207624.5).

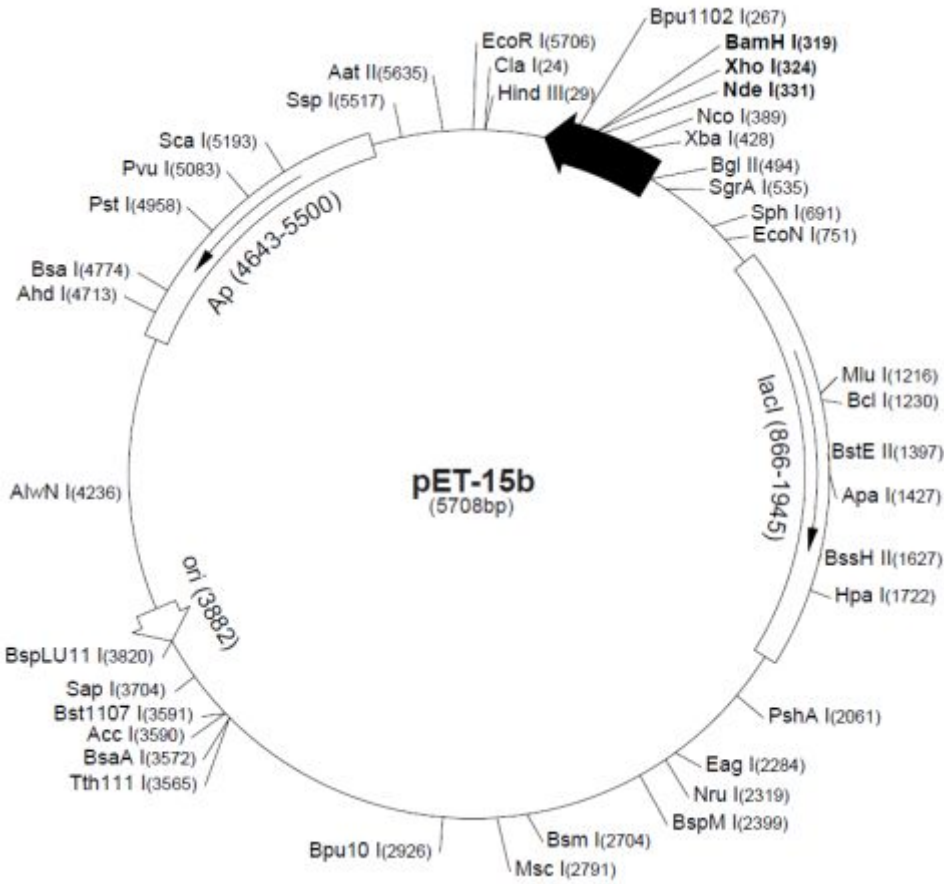

Fig. 13. The map of pET-15b vector. This vector is composed with N-terminal His·Tag, T7 promoter, lac operator gene, thrombin site, and three cloning sites. T7 expression region is particularly reversed on the circular map. Cloning and protein expression are selection in DH5α and BL21(DE3), respectively.

CTCGACCCTGGATTGCAGCCGGGCAACTTTTCCCCGGACGAGGCAGGGGCGCA
GCTTTTCGCTGAAAGCTATAACTCGAGTGCCGAGGTGGTGATGTTCCAGAGCAC
CGTGGCCAGTTGGGCGCACGACACCAACATCACGGAGGAGAACGCGCGACGCC
AGGAGGAAGCGGCCCTGGTCAGCCAGGAGTTTGCAGAGGTCTGGGGCAAGAA
GGCCAAGGAGTTGTATGAGTCCATTTGGCAGAACTTTACTGACTCAAAGCTGCG
AAGGATCATCGGATCTATTCGGACCCTAGGACCTGCCAATCTGCCCCTGGCCCA
GCGGCAGCAGTACAACTCTCTGCTAAGCAACATGAGCAGAATCTACTCCACTG
GCAAGGTCTGCTTCCCCAACAAGACTGCCACCTGCTGGTCCCTGGACCCAGAGC
TCACCAACATCCTGGCTTCCTCACGAAGCTATGCCAAGTTGTTGTTTGCCTGGG
AGGGCTGGCATGATGCTGTGGGTATCCCACTGAAACCCCTCTATCAAGACTTTA
CTGCCATCAGTAACGAAGCCTACAGACAAGACGACTTCTCAGACACAGGAGCC
TTCTGGCGCTCCTGGTATGAGTCCCCCTCCTTTGAGGAGAGTCTGGAACATATC
TACCACCAACTAGAGCCCCTCTACCTGAACCTCCATGCCTACGTCCGCCGCGCA
CTGCACCGCCGCTATGGGGACAAATACGTCAATCTCAGGGGGCCTATTCCTGCC
CATTTGCTGGGAGACATGTGGGCCCAGAGCTGGGAGAACATCTACGACATGGT
AGTGCCTTTCCCAGACAAACCCAACCTCGATGTCACCAGTACAATGGTACAGA
AGGGCTGGAACGCCACACACATGTTCCGGGTATCAGAGGAATTCTTCACCTCGC
TGGGGCTCTCACCCATGCCTCCTGAGTTCTGGGCGGAGTCAATGCTGGAGAAAC
CAACGGATGGACGGGAGGTGGTGTGCCACGCCTCTGCCTGGGACTTCTACAAC
CGGAAGGACTTCCGGATTAAGCAATGCACACGGGTCACGATGGAACAGCTGGC
CACAGTACACCACGAGATGGGCCACGTGCAGTACTACCTCCAGTACAAGGACC
TGCACGTCTCTCTGCGTAGAGGTGCCAACCCTGGCTTCCATGAGGCCATTGGGG
ATGTGCTTGCACTCTCCGTCTCTACCCCTGCACATCTGCACAAAATCGGCCTACT
GGACCATGTTACCAATGACATAGAGAGTGACATCAATTACCTGCTAAAGATGG
CCCTAGAGAAAATCGCCTTCTTGCCCTTTGGCTACCTGGTGGACCAGTGGCGTT
GGGGGGTCTTCAGTGGACGGACCCCACCCTCTCGCTACAACTTCGACTGGTGGT

ATCTTCGAACCAAGTATCAGGGGATCTGCCCACCAGTTGCCCGGAATGAAACC
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TACTTCGTGAGCTTTGTGCTGCAGTTCCAGTTCCATCAAGCACTGTGCAAGGAG
GCAGGCCACCAGGGCCCACTACACCAGTGTGACATCTACCAGTCCGCCCAGGC
GGGGGCCAAGCTCAAGCAGGTGTTGCAGGCTGGCTGCTCCAGGCCCTGGCAGG
AGGTACTGAAGGACCTGGTAGGCTCAGATGCCCTGGATGCCAAGGCACTGCTG
GAGTACTTCCAACCGGTCAGCCAGTGGCTGGAAGAGCAGAATCAGCGGAATGG
CGAAGTCCTAGGCTGGCCAGAGAATCAGTGGCGTCCACCGTTACCCGACAACT
ATCCAGAGGGCATTGACCTAGAGACTGATGAAGCCAAGGCTGACAGGTTCGTG
GAAGAGTATGACCGGACAGCCCAAGTGTTGTTGAACGAGTACGCAGAGGCCAA
CTGGCAATATAACACCAACATTACCATAGAGGGCAGCAAGATCCTGCTTGAGA
AAAGCACGGAGGTATCCAATCACACCCTGAAATATGGCACCCGGGCCAAGACA
TTTGATGTGAGCAACTTTCAAAACTCTTCCATCAAGCGGATCATAAAGAAGCTT
CAGAACCTGGACCGGGCAGTGCTGCCTCCCAAGGAATTAGAAGAGTACAACCA
GATCCTGCTAGACATGGAGACAACTTACAGCTTATCCAACATTTGCTACACAAA
TGGCACTTGTATGCCCCTGGAACCTGATCTAACAAACATGATGGCCACATCCCG
GAAATATGAAGAATTGCTATGGGCATGGAAGAGCTGGAGAGACAAGGTGGGG
AGAGCCATCCTTCCTTTTTTCCCAAAGTATGTGGAGTTCTCCAACAAGATTGCC
AAGCTCAATGGCTACACGGATGCAGGGGATTCATGGAGATCCTTATACGAGTC
TGACAACCTGGAGCAAGACCTGGAAAAACTGTACCAGGAGCTGCAGCCACTCT
ACCTGAACCTGCATGCCTATGTGCGTCGTTCCCTGCACCGCCACTATGGGTCCG
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CGCAGACCTGGTCCAACATCTATGATTTGGTGGCGCCCTTCCCTTCCGCCCCCA
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ATTTAAGGAAGCTGACAATTTCTTTACCTCCCTGGGGCTGTTACCTGTGCCCCCT
GAGTTCTGGAACAAGTCGATGTTAGAGAAGCCCACCGATGGAAGGGAGGTGGT

GTGCCATCCCTCAGCCTGGGACTTCTACAACGGCAAGGACTTCAGGATCAAGC
AGTGTACCTCTGTGAACATGGAGGACTTGGTGATAGCGCACCACGAAATGGGC
CACATCCAGTATTTCATGCAGTACAAAGACTTACCCGTGACTTTCCGGGAGGGT
GCCAACCCTGGTTTTCATGAAGCTATTGGAGATATAATGGCTCTCTCAGTGTCT
ACCCCCAAGCATCTATACAGTCTCAACCTGCTTAGCACTGAGGGCAGTGGCTAC
GAGTATGACATCAACTTTCTAATGAAGATGGCCCTCGACAAGATCGCCTTTATC
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ACCAAGGAGAACTATAACCAGGAGTGGTGGAGCCTCAGGCTGAAGTATCAGGG
TCTGTGCCCCCCAGTGCCAAGATCCCAAGGTGACTTTGACCCAGGGTCCAAGTT
CCACGTTCCTGCGAACGTGCCATACGTCAGGTACTTTGTCAGCTTCATCATCCA
GTTCCAGTTCCACGAGGCGCTGTGTCGCGCAGCCGGGCACACGGGTCCCCTGCA
CAAGTGTGACATCTACCAATCCAAGGAAGCAGGGAAGCTCCTGGCGGATGCCA
TGAAGCTGGGCTACAGTAAGCCGTGGCCAGAGGCCATGAAGCTGATCACAGGC
CAGCCTAACATGTCAGCCTCCGCCATGATGAATTACTTCAAGCCACTGACAGAA
TGGCTCGTCACCGAGAACAGGAGACATGGAGAGACACTGGGCTGGCCGGAGTA
CAACTGGGCGCCAAACACCGCTCGCGCAGAAGGCTCCACCGCAGAGTCCAACC
GCGTCAATTTCCTGGGCCTGTACCTGGAGCCACAGCAGGCCCGCGTGGGCCAGT
GGGTGCTGCTCTTCCTGGGCGTCGCCCTGCTCGTGGCCACCGTGGGTCTCGCCC
ATCGGCTCTACAACATCCGTAACCATCACAGCCTCCGCCGGCCCCACCGTGGGC
CCCAGTTTGGGTCTGAGGTGGAGCTCAGACAC

Fig. 14. DNA Sequence of ACE mature protein. DNA size is 3.831 bp. Assession number is NM-207624.5. mature protein start is 140 nucleotide and stop is 3,973 nucleotide.

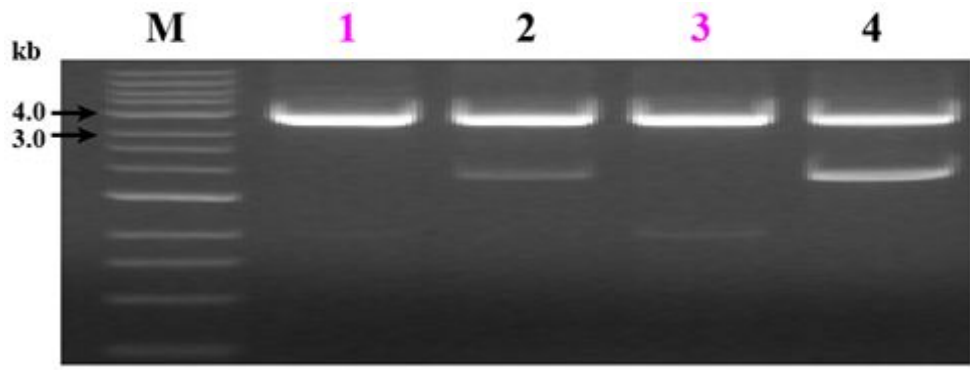

Fig 15. RT-PCR result of ACE. ACE DNA was amplified by forward and reverse primers. 1 and 3 were used ACE-F7-8 and ACE-R7-9. 2 and 4 were used ACE-F7-8 and ACE-R7-10. M is 1,000 bp marker.

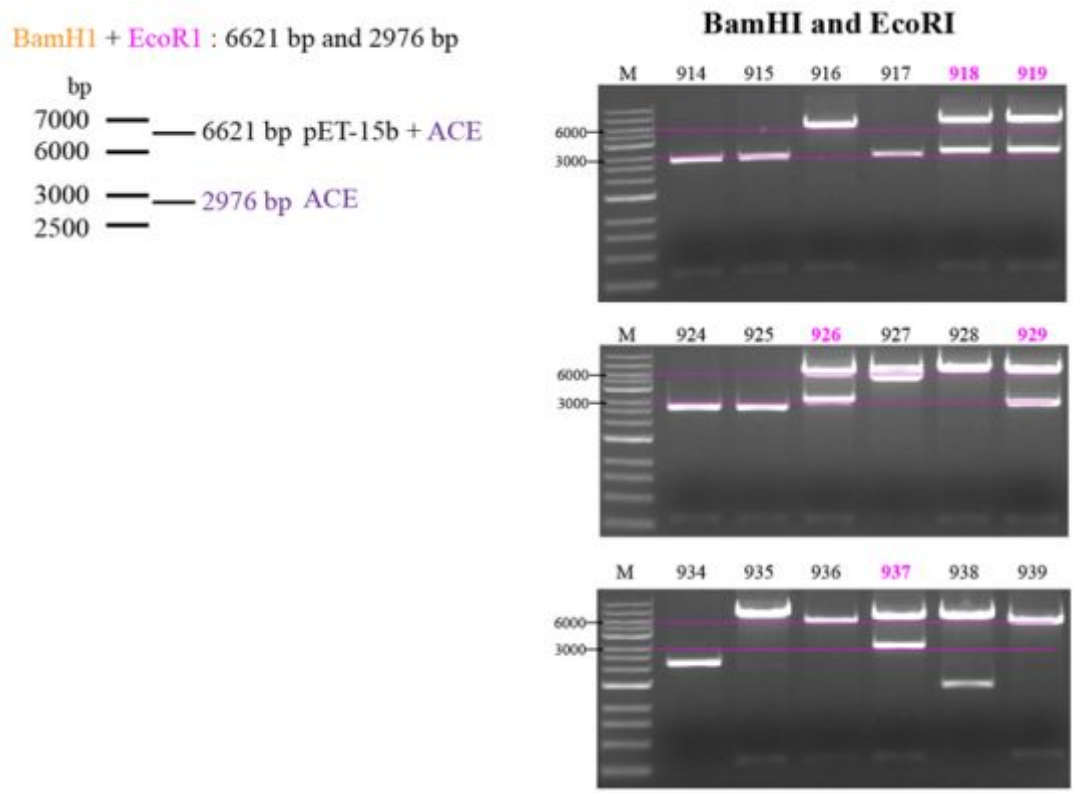

Fig. 16. The expected band and a real band by BamH I and EcoR I. Left image is the expected band by BamH I and EcoR I. Right image is a real band by BamH I and EcoR I.

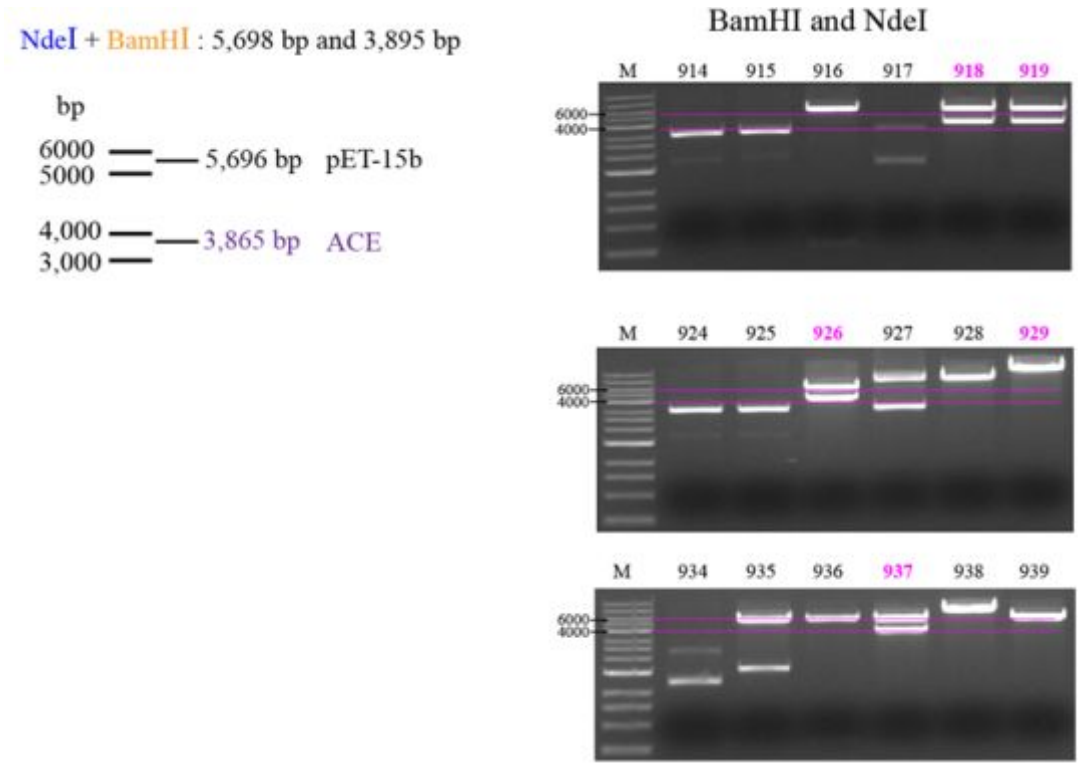

Fig. 17. The expected band and a actual band by BamH I and Nde I. Left image is the expected band by BamH I and Nde I. Right image is a real band by BamH I and Nde I.

Fig. 18. The map of construction ACE gene. The construct map for ACE in vector pET-15b.

```
pET15b-ACE      HMENLYFQGMLDPGLQPGNFSPDEAGAQLFAESYNSSAEVVMFQSTVASWAHDTNITEEN
Mouse ACE                 LDPGLQPGNFSPDEAGAQLFAESYNSSAEVVMFQSTVASWAHDTNITEEN

pET15b-ACE      ARRQEEAALVSQEFAEVWGKKAKELYESIWQNFTDSKLRRIIGSIRTLGPANLPLAQRQQ
Mouse ACE       ARRQEEAALVSQEFAEVWGKKAKELYESIWQNFTDSKLRRIIGSIRTLGPANLPLAQRQQ

pET15b-ACE      YNSLLSNMSRIYSTGKVCFPNKTATCWSLDPELTNILASSRSYAKLLFAWEGWHDAVGIP
Mouse ACE       YNSLLSNMSRIYSTGKVCFPNKTATCWSLDPELTNILASSRSYAKLLFAWEGWHDAVGIP

pET15b-ACE      LKPLYQDFTAISNEAYRQDDFSDTGAFWRSWYESPSFEESLEHIYHQLEPLYLNLHAYVR
Mouse ACE       LKPLYQDFTAISNEAYRQDDFSDTGAFWRSWYESPSFEESLEHIYHQLEPLYLNLHAYVR

pET15b-ACE      RALHRRYGDKYVNLRGPIPAHLLGDMWAQSWENIYDMVVPFPDKPNLDVTSTMVQKGWNA
Mouse ACE       RALHRRYGDKYVNLRGPIPAHLLGDMWAQSWENIYDMVVPFPDKPNLDVTSTMVQKGWNA

pET15b-ACE      THMFRVSEEFFTSLGLSPMPPEFWAESMLEKPTDGREVVCHASAWDFYNRKDFRIKQCTR
Mouse ACE       THMFRVSEEFFTSLGLSPMPPEFWAESMLEKPTDGREVVCHASAWDFYNRKDFRIKQCTR

pET15b-ACE      VTMEQLATVHHEMGHVQYYLQYKDLHVSLRRGANPGFHEAIGDVLALSVSTPAHLHKIGL
Mouse ACE       VTMEQLATVHHEMGHVQYYLQYKDLHVSLRRGANPGFHEAIGDVLALSVSTPAHLHKIGL

pET15b-ACE      LDHVTNDIESDINYLLKMALEKIAFLPFGYLVDQWRWGVFSGRTPPSRYNFDWWYLRTKY
Mouse ACE       LDHVTNDIESDINYLLKMALEKIAFLPFGYLVDQWRWGVFSGRTPPSRYNFDWWYLRTKY

pET15b-ACE      QGICPPVARNETHFDAGAKFHIPNVTPYIRYFVSFVLQFQFHQALCKEAGHQGPLHQCDI
Mouse ACE       QGICPPVARNETHFDAGAKFHIPNVTPYIRYFVSFVLQFQFHQALCKEAGHQGPLHQCDI

pET15b-ACE      YQSAQAGAKLKQVLQAGCSRPWQEVLKDLVGSDALDAKALLEYFQPVSQWLEEQNQRNGE
Mouse ACE       YQSAQAGAKLKQVLQAGCSRPWQEVLKDLVGSDALDAKALLEYFQPVSQWLEEQNQRNGE

pET15b-ACE      VLGWPENQWRPPLPDNYPEGIDLETDEAKADRFVEEYDRTAQVLLNEYAEANWQYNTNIT
Mouse ACE       VLGWPENQWRPPLPDNYPEGIDLETDEAKADRFVEEYDRTAQVLLNEYAEANWQYNTNIT
```

Fig. 19. Sequence alignment analysis of pET15b-ACE and mouse ACE protein sequences. Each protein indicated the left of the alignment. The enzyme restriction site at each end of pET15b-ACE is red letter. The amino acid HM are Nde I site and GS are BamH1 site. The amino acid ENLYFQGM are TEV site.

```
pET15b-ACE      IEGSKILLEKSTEVSNHTLKYGTRAKTFDVSNFQNSSIKRIIKKLQNLDRAVLPPKELEE
Mouse ACE       IEGSKILLEKSTEVSNHTLKYGTRAKTFDVSNFQNSSIKRIIKKLQNLDRAVLPPKELEE

pET15b-ACE      YNQILLDMETTYSLSNICYTNGTCMPLEPDLTNMMATSRKYEELLWAWKSWRDKVGRAIL
Mouse ACE       YNQILLDMETTYSLSNICYTNGTCMPLEPDLTNMMATSRKYEELLWAWKSWRDKVGRAIL

pET15b-ACE      PFFPKYVEFSNKIAKLNGYTDAGDSWRSLYESDNLEQDLEKLYQELQPLYLNLHAYVRRS
Mouse ACE       PFFPKYVEFSNKIAKLNGYTDAGDSWRSLYESDNLEQDLEKLYQELQPLYLNLHAYVRRS

pET15b-ACE      LHRHYGSEYINLDGPIPAHLLGNMWAQTWSNIYDLVAPFPSAPNIDATEAMIKQGWTPRR
Mouse ACE       LHRHYGSEYINLDGPIPAHLLGNMWAQTWSNIYDLVAPFPSAPNIDATEAMIKQGWTPRR

pET15b-ACE      IFKEADNFFTSLGLLPVPPEFWNKSMLEKPTDGREVVCHPSAWDFYNGKDFRIKQCTSVN
Mouse ACE       IFKEADNFFTSLGLLPVPPEFWNKSMLEKPTDGREVVCHPSAWDFYNGKDFRIKQCTSVN

pET15b-ACE      MEDLVIAHHEMGHIQYFMQYKDLPVTFREGANPGFHEAIGDIMALSVSTPKHLYSLNLLS
Mouse ACE       MEDLVIAHHEMGHIQYFMQYKDLPVTFREGANPGFHEAIGDIMALSVSTPKHLYSLNLLS

pET15b-ACE      TEGSGYEYDINFLMKMALDKIAFIPFSYLIDQWRWRVFDGSITKENYNQEWWSLRLKYQG
Mouse ACE       TEGSGYEYDINFLMKMALDKIAFIPFSYLIDQWRWRVFDGSITKENYNQEWWSLRLKYQG

pET15b-ACE      LCPPVPRSQGDFDPGSKFHVPANVPYVRYFVSFIIQFQFHEALCRAAGHTGPLHKCDIYQ
Mouse ACE       LCPPVPRSQGDFDPGSKFHVPANVPYVRYFVSFIIQFQFHEALCRAAGHTGPLHKCDIYQ

pET15b-ACE      SKEAGKLLADAMKLGYSKPWPEAMKLITGQPNMSASAMMNYFKPLTEWLVTENRRHGETL
Mouse ACE       SKEAGKLLADAMKLGYSKPWPEAMKLITGQPNMSASAMMNYFKPLTEWLVTENRRHGETL

pET15b-ACE      GWPEYNWAPNTARAEGSTAESNRVNFLGLYLEPQQARVGQWVLLFLGVALLVATVGLAHR
Mouse ACE       GWPEYNWAPNTARAEGSTAESNRVNFLGLYLEPQQARVGQWVLLFLGVALLVATVGLAHR

pET15b-ACE      LYNIRNHHSLRRPHRGPQFGSEVELRHSGS
Mouse ACE       LYNIRNHHSLRRPHRGPQFGSEVELRHS
```

Fig. 20. Alignment analysis of pET15b-ACE and mouse ACE amino acid sequences (continued on previous page).

Ligand docking and binding site analysis with PyMOL, PyRx and Autodock/Vina

Designing anti-hypertension drug targeted ACE or ACE binding-domain of tartaric acid is very promising. However the ACE-binding site of tartaric acid still debatable, then we analyzed the binding site based on their interaction using molecular docking. Since the three dimensions (3D) structure of tartaric acid was still not available yet then we used 3D model of tartaric acid to determine it's binding to ACE. Furthermore, we used the valid model to analyze ACE-binding site of tartaric acid based on the flexible docking. The result showed that the lowest energy binding of ACE-tartaric acid complexis -4.4 kcal/mol (Captopril -5.4 kcal/mol), and ACE bind to substrate binding domain of tartaric acid.(Fig. 22)

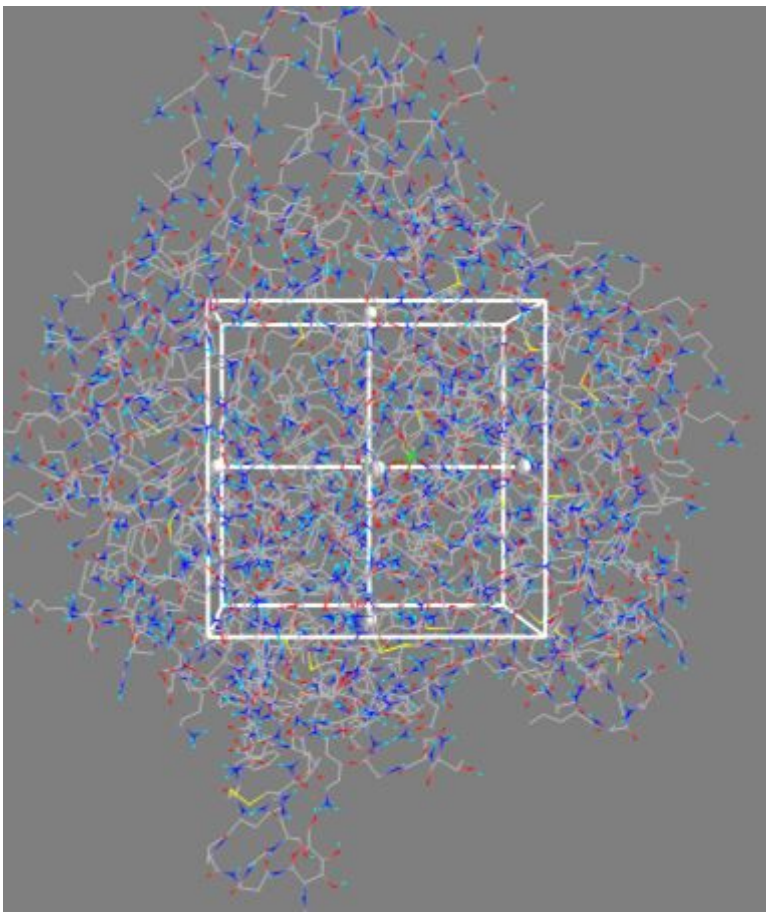

Fig. 21. 3D model of ACE and binding site (white box)

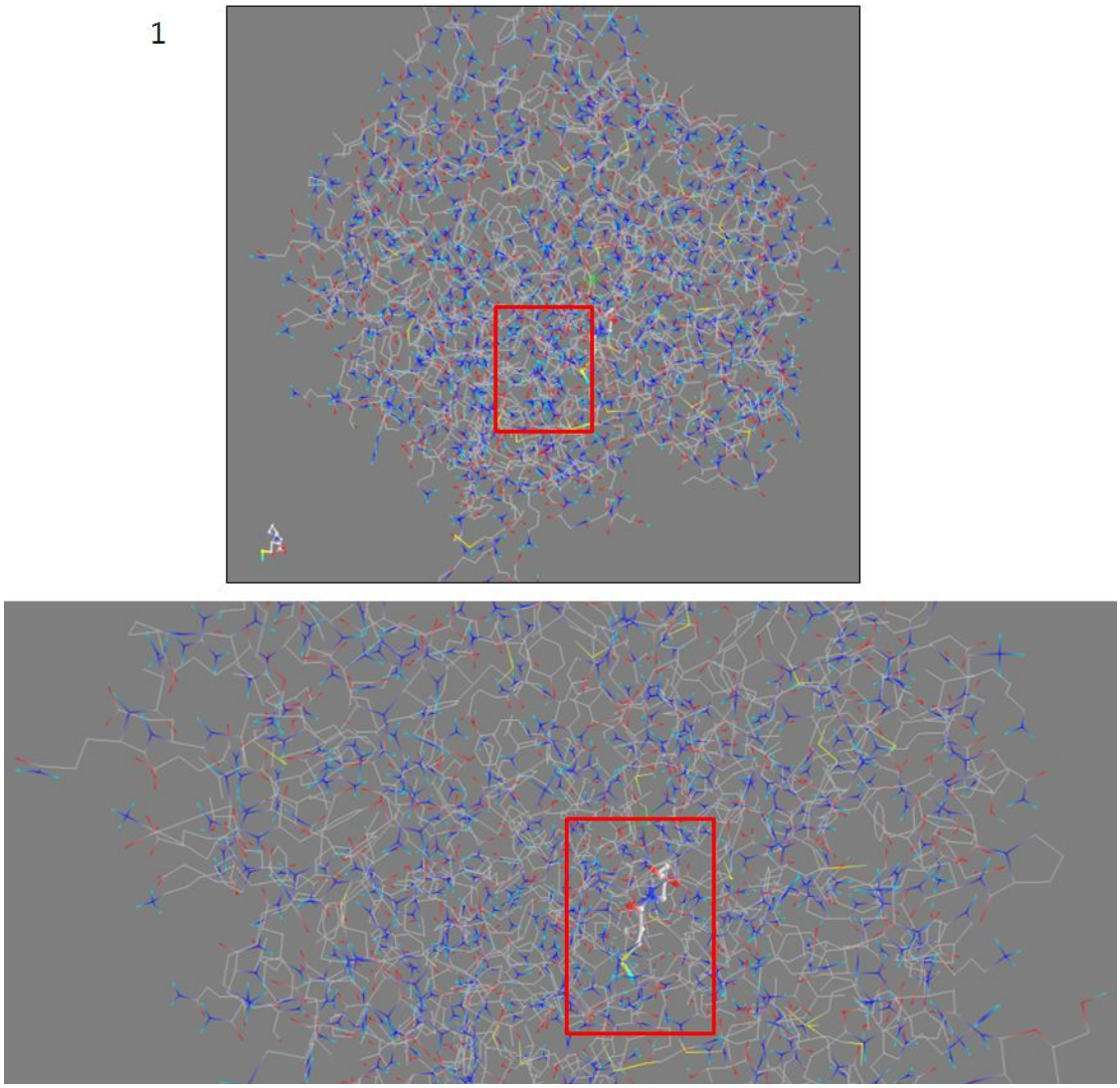

Fig. 22-1. : ACE-Captopril complex. ACE binds to captopril (red box) on substrate binding domain

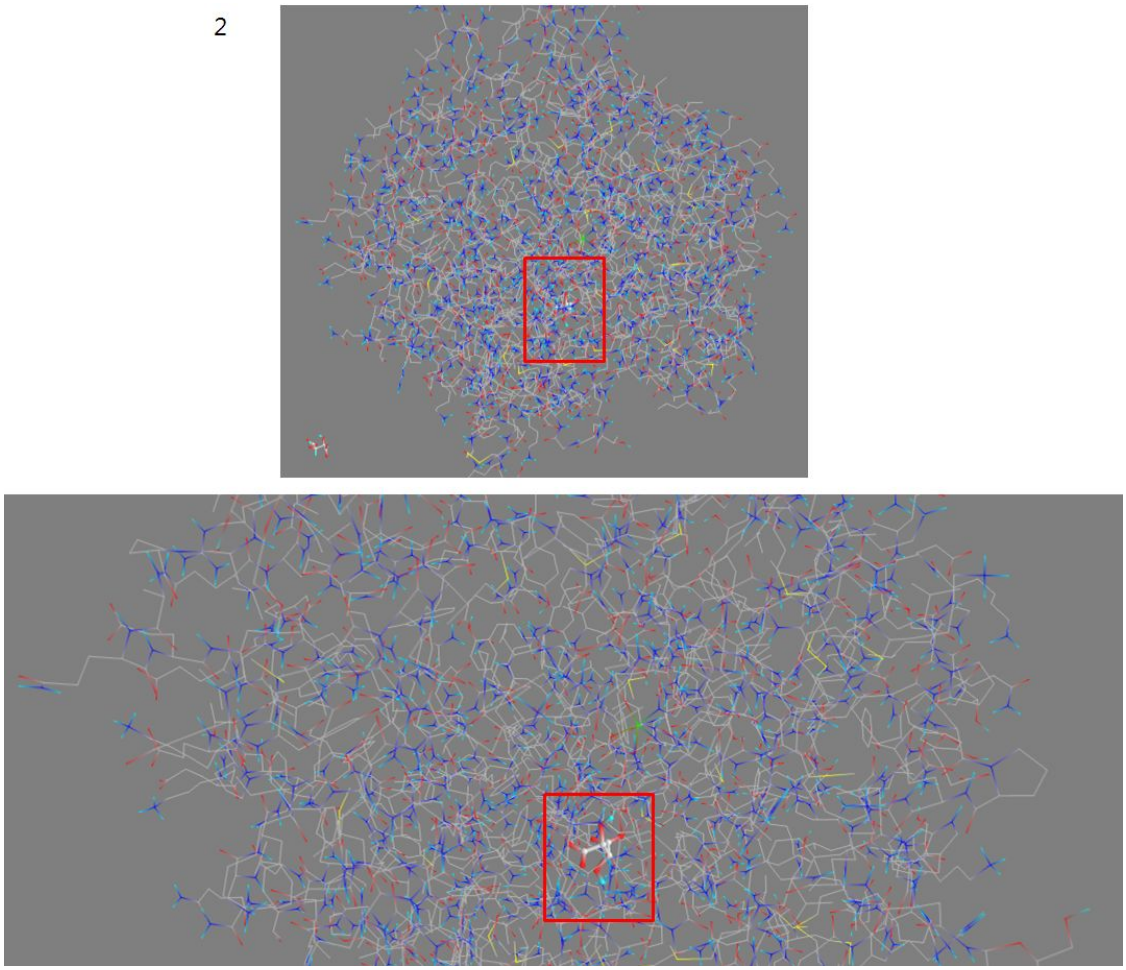

Fig. 22-2. : ACE-Tartaric acid complex. ACE binds to tataric acid (red box) on substrate binding domain

DISSCUSION

ACE inhibition would be expected to prevent the formation of the vasoconstrictory (hypertensive) agent angiotensin II and to potentiate the vasodilatory (hypotensive) properties of bradykinin leading to a concerted lowering of blood pressure. Inhibitors of ACE are therefore widely used in therapy for hypertension, heart failure, myocardial infarction and diabetic nephropathy (Choi, H.S., et al, 2001). In this study, to determine the tartaric acid for ACE inhibition performed on ACE inhibitory assay using variety buffer and mixture of optical isomers of tartaric acid (DL-, D-, L- and Meso form). When using tris-zinc buffer, ACE inhibition was stability and higher inhibition then other buffer. From the result, all types of tartaric acid showed the high inhibitory activity. particularly in mixture of L-form and Meso form, highest inhibitory activity. Elevated blood pressure is often treated with commercial drugs that inhibit ACE, such as captopril and enalapril, but ACE inhibitory peptides may provide an alternative treatment. The tartaric acids were similarly in ACE inhibition than captopril, tartaric acid is eaten daily. And identified ACE-tartaric acid complex. Here we found tartaric acid from Zinc database that potentially abrogate ACE-ANG I interaction using Virtual Screening. Daily use of food that contains tartaric acid with potent ACE inhibitory activity could be effective in maintaining blood pressure at a healthy level. These tartaric acids are less potent than synthetic ones, but they do not exhibit side effects (Balti, R., et al, 2015). For development of the effective preparation of the tartaric acid, further work is needed to constructs for structure study of ACE pET-15b vector including the His·tag and T7 promoter was used for crystal structures of ACE in complex with tartaric acid. (Houard, X., et al, 1998) and inhibitor binding assay and expression of angiotensin II receptor, type 1 and endothelial nitric oxide synthase 3 (eNOS) in mouse endothelial cells. In order to investigate the antihypertesion activity of the purified compound , cDNA expression of Angiotensi II receptor, type 1 (AT1 receptor)

and endothelial nitric oxide synthase 3 (eNOS) was identified by RT-PCR in SVEC 4-10. To identify expression of AT1 receptor and eNOS gene in SVEC 4-10, group of cells were exposed to high glucose concentration for a shorter period of 6 h. Vidotti, D., et al, 2004)

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감사의 글

2012년부터 벌써 3년이란 시간동안 실험실에서 보내고 이렇게 석사학위를 받게 되었습니다. 석사학위를 받기까지 지도해주시고 도와주신 저희 정현숙 교수님께 진심으로 감사드립니다. 지도교수와 제자의 사이가 아닌 마치 어머니 같이 따뜻하게 대해주시고 혼내시면서 지도해주셔서 항상 교수님께 감사할 따름이 였습니다. 제가 박사과정으로 진학을 하지 않고, 졸업을 결정하였을 때도 제 의견을 존중해주시고 여러 가지 조언을 해주셨을 때 정말 교수님 밑에서 석사과정을 보낸 것이 큰 행운이 였다는 것을 느꼈습니다. 더 오랜 시간 교수님과 보내지 못하고 나가는 점이 죄송스러울 뿐입니다.
또, 학위논문을 심사해주신 저희 실험실 선배이시자 항상 저를 반갑게 맞아주셨던 박윤경 교수님께 감사의 뜻을 전합니다. 부족한 제가 미생물 실험을 하였을 때, 실험에 도움을 주셨던 점 잊지 않겠습니다. 학위논문을 심사해주신 김호중 교수님께도 감사의 말씀을 전합니다. 저희 실험실에 화학 관련의 자문을 해주시고 도와주셔서 감사합니다.

3년이란 실험실 생활에 제 실험의 또 다른 멘토가 되어주신 박재영 박사님 항상 실험에 대한 의견과 궁금한 점을 여쭈어 볼 수 있어서 든든한 버팀목이 되었습니다. 나이는 적지만 저보다 더 어른 같은 실험실 선배인 범기. 나이 많은 후배가 들어와서 대하기 힘들었을 텐데 먼저 다가와주고 실험에 관해 거침없이 가르쳐줘서 고마웠어.
실험실 들어오기 전부터 항상 붙어 다녔던 웅이. 벌써 8년이란 시간을 붙어 다녔는데 이제 떨어져 지내겠다. 안 볼 건 아니니까 넌 실험실 생활 잘 할 테니 좋은 결과 내길 바랄게. 실험실 첫 후배인 다빈이. 처음엔 같이 실험했는데 네가 따로 프로젝트를 맡고나서는 네가 나보다 더 열심히 하고 더 잘하더라. 결과 안 나온다고 풀 죽지말고 곧 좋은 결과 나올 거야. 그리고 실험실 마지막 후배인 지은이. 항상 실험결과에 따라서 웃기도하고 우울해하기도 하던 네 모습보면 저게 과학자의 모습인가 라고 생각하기도 했어. 항상 열심히 하고 결과가 잘 안 나오면 여기저기 물어보고 의견을 구하는 모습이 보기 좋았어, 우울해하지고 항상 웃으렴. 우리 실험실 모두 힘내서 좋은 결과 냈으면 합니다. 실험실에 나가면서 다른 곳에서도 초심을 잃지 않고 새로운 시작으로 잘 헤쳐나가겠습니다. 다들 감사합니다.

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