Antihypertensive properties of lemongrass

ASSIGN BUSTER

Hypertension or high blood pressure is defined as the sustained increase in blood pressure and is a common disorder leading to several chronic diseases. The number of people considered to be suffering from hypertension depends on levels of normality for blood pressure given by different health organizations. Recently, hypertension affects 20-45% of the world's population and nearly 50-60% of elderly people and is a major risk factor in cardiovascular disease and other related complications (Hartmann and Meisel, 2007; Yang et al., 2004). It is likely to increase to 29% by 2025. There are many factors considered why people develop hypertension. Changes in lifestyle, physical exercises, intake of healthy diets are some common issues associated with reducing the risk of hypertension (Balasuriya and Rupasinghe, 2011). Moreover, eating foods with high dietary proteins helps in reducing the chance of having high blood pressure.

The pathogenesis of hypertension could be due to many reasons. For example, increased activity of renin angiotensin aldosterone system (RAAS), kalikerenin kinin system and sympathetic nervous system, and genetic influence are specified (Balasuriya and Rupasinghe, 2011). In the mechanism of hypertension, Angiotensin converting enzyme (ACE) converts the precursor angiotensin I into the potent vasoconstrictor angiotensin II, which is the peptide responsible in triggering blood pressure increasing mechanism, and inactivates the vasolidator bradykinin. The role of ACE inhibitory peptides is to stop the said process, making the peptides antihypertensive (Kuba et al., 2004).

Different types of natural food derived compounds have been investigated on their ACE inhibitory properties. Numerous studies performed in

spontaneously hypertensive rats as well as hypertensive people gave a positive result about the antihypertensive effects of food-derived ACE-inhibitors. Thus, results showed that hypertension can be prevented by the antihypertensive effects of ACE inhibitory peptides (Erdmann, 2008).

Food proteins can be divided into three categories as animal-derived, plant-derived and microorganism-derived proteins. Plant-derived proteins have also been identified from different sources. Another group of natural compounds which are identified as potential ACE inhibitors are secondary metabolites produced in plants (Balasuriya and Rupasinghe, 2011). Plants are renewable sources of food protein. Seed proteins are mainly used for the production of protein-rich foods. However, due to mass production, the green parts of plants are potentially sources of valuable protein (Yang et al., 2003). One of the greatest constituents of biomass proteins is ribulose biphosphate carboxylase/oxygenase (Rubisco), which catalyzes the primary step in photosynthetic CO2 fixation. Rubisco, is the most abundant protein on earth and is an important source of peptides because of its amino acid composition. It was hypothesized that rubisco may have importance in prophylaxis of hypertension (Yang et al., 2004).

The main focus of the study is the Lemon grass (Cymbopogon citratus). The plant is a native herb from India and is cultivated in other tropical and subtropical countries. It can be planted on the contour on steep banks to control soil erosion and it is also useful as a barrier to running grasses around vegetable gardens. Lemon grass is consumed as an aromatic drink and in traditional cuisine for its lemon flavor (Hindumathay, 2011). It is also

used as traditional folk medicine in the treatment of nervous, gastrointestinal disturbances fevers and hypertensions (Oloyede, 2009).

Objectives

The general objective of this study is to determine the presence of ACE inhibitory and antibacterial peptides from the leaves of Lemon grass (Cymbopogon citratus).

The specific objectives of this study are:

- to extract the total proteins from the lemon grass leaves;
- to digest the total leaf proteins using trypsin and chymotrypsin;
- to purify the tryptic and chymotryptic digests using RP-HPLC;
- to determine the ACE inhibitory and antibacterial activities of the RP-HPLC purified fractions; andto IC50 of the ACE inhibitory peptides.

REVIEW OF RELATED LITERATURE

Lemon Grass

Cymbopogon citratus, commonly known as Lemon grass or Tanglad in the Philippines is a native herb from India and is also cultivated in other tropical and subtropical countries for its medicinal and other uses (Hinumathay, 2011). It is an aromatic perennial tall grass with rhizomes and densely tufted fibrous roots (Oloyede, 2009). Its leaves grow to a length of up to 1 meter, about 1-1. 5 cm wide, scabrous, flate, long acuminate and smooth and grow in dense clump up to 2 meters in diameters. The plant needs a warm, humid climate. Lemon grass grows well in sandy soils with adequate drainage. The propagation is by root or plant division. In folk medicine, lemon grass is used as fragrance and flavoring for a wide variety of ailments. Freshly cut and

partially dried leaves are used medicinally and are the source of the essential oil (Aftab et. al., 2011).

Lemon grass contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol (Hinumathay, 2011). Like other members of the genus, citratus, yields citral, a volatile oil with strong lemon fragrance. It is used in manufacture of perfumes, colored soaps and synthesis of Vitamin A (Nwachukwu, 2008).

Rubisco

The chloroplast enzyme, Ribulose-1, 5-bisphosphate carboxylase/oxygenase, most commonly known as Rubisco, is a bifunctional enzyme that is used in the Calvin Cycle to catalyze the first major step of carbon fixation and oxygenation of ribulose bisphosphate in the photorespiratory pathway (Roy, 1989). Rubisco is thought to be the most abundant protein in the world and comprises up to 50 % of all soluble proteins in plants and it is a very large and complex protein. It is also an excellent model for protein assembly studies due to some reasons: (1) the structure is known; (2) Rubisco is very abundant; (3) Prokaryotic Rubisco can be studied in E. coli using cloned genes from cyanobacteria and autotrophic and chemoautrophic bacteria; and (4) higher plant Rubisco assembly can be studied in chloroplast extracts (Chatterjee and Basu, 2011).

Rubisco has its own limitations. Most enzymes typically can carry out thousands of chemical reactions each second. In contrast, Rubisco is notoriously inefficient as a catalyst for the carboxylation of RuBP, being able to fix only about 3 CO2 molecules per second. This slow and inefficient

activity can be attributed to several factors. Some of these factors are, the competitive inhibition by O2, the dead-end inhibition by RuBP, inactivation by loss of carbamylation, Rubisco’s multi-step mechanism and many more (Chatterjee and Basu, 2011).

Bioactive Peptides

It is now well established during gastrointestinal digestion and fermentation of food materials with lactic acid bacteria physiologically active peptides are produced from several food proteins. Biologically active peptides are food-derived peptides and are inactive within the sequence of their parent protein. They can be released through enzymatic hydrolysis (Korhonen, 2006). And upon release, they exert, beyond their nutritional value, a physiological hormone-like effects in humans based on their inherent amino acid composition and sequence (Erdmann et. al., 2008). Biologically active peptides are composed of 2-20 amino acid residues per molecule, but many consist of more than 20 amino acids in other cases. Bioactive peptides are produced using dietary proteins by means of the following four mechanisms; (1) during the fermentation of food using proteolytic starter cultures; (2) during the manufacture if protein hydrosylates; (3) as a result of the degradation of dietary proteins by digestive enzymes in vivo; or (4) as a result of the enzymatic action of digestive enzymes in vitro. And many peptides are known to reveal multifunctional properties. Because of their potential in enhancing health and safety profiles, they may be used as components in functional foods or nutraceuticals.

ACE Inhibitory Peptides

ACE or kininase II was originally isolated from horse blood in 1956 as a hypertension-converting enzyme. It is a monomeric glycoprotein that is distributed in many tissues and biological fluids (Guang and Pihillips, 2009). The Angiotensin I-converting enzyme (ACE, dipeptidyl carboxypeptidase I, or kininase II) is a multifunctional, zinc-containing enzyme that is located in different tissues and plays an important role in blood pressure regulation and fluid salt balance in mammals (Kim et. al., 2004). The renin-angiotensin system (RAS) plays an important role in blood pressure regulation. Renin produces decapitate angiotensin I from Angiotensinogen. The angiotensin I-converting enzyme (ACE) catalyzes the formation of vasopressor angiotensin II by cleaving dipeptide from the C-terminal of angiotensin I in the vascular wall (Nakuno et. al., 2006).

ACE regulates the balance between RAS and kallikrein-kinin system because it cleaves the C-terminal dipeptide from Angiotensin I and bradykinin and a number of other small peptides which lack a penultimate proline residue. The RAS regulates the blood pressure. There are factors that stimulate the secretion of renin by the juxtaglomerular cell, which are reduced sodium delivery at the macula densa, decreased renal perfusion pressure, and sympathetic activation. The inactive decapeptide Angiotensin I is cleaved by the renin from the prohormone angiotensinogen, a nonhibiting member of the serpin family of serin protease inhibitor. Angiotensin II, a potent vasoconstrictor, which acts directly on vascular smooth cell, is then cleaved by angiotensin I by the action of ACE. Volume expansion through sodium retention (via aldosterone and renal vasoconstriction) and fluid retention (via

antidiuretic hormone) is caused by angiotensin II. It also promotes migration, proliferation and hypertrophy at the cellular level.

ACE catalyzes the degradation of bradykinin, in which, in specific tissues or organs, it causes different functions like smooth muscle contraction and many more. However, promotion of vasodilation by stimulating the production of arachidonic acid metabolites, nitric oxides, and endothelium-derived hyperpolarizing factor in vascular endothelium are supported by bradykinin. It also causes natriuresis in the kidney through direct tubular effects. The B2 receptor serves as the venue for the most of the physiological effects of bradykinin.

The balance between the vasodilatory and natriuretic properties of bradykinin and the vasoconstrictive and salt-retentive properties of Angiotensin II is regulated by ACE. It alters this balance by decreasing the formation of Angiotensin II and the degradation of bradykinin (Brown and Vaughan, 1998).

MATERIALS AND METHODS

Preparation of Lemon grass leaf protein

Fresh Lemon grass will be obtained from a local supermarket.

The method described by Satoh et al. (year) with slight modification will be used to extract the lemon grass total leaf protein. Fresh lemon grass leaves will be homogenized with approximately equal amounts of aqueous 0. 003 N NaOH by weight at 5 oC in a Waring blender. The homogenate obtained (pH 11) will be filtered through two pieces of gauze and the filtrate will be centrifuged at 13, 500 x g for 50 min at 5 oC with the use of SRC20B

centrifuge from Hitachi (Japan). The supernatant will be filtered through Whatman No. 2 filter paper, and will be adjusted to a pH of about 4. 5 (final pH) with 5% acetic acid to produce protein precipitate. The precipitate obtained will be washed successively with acetic acid, ethanol, and diethyl ether, on a glass filter until the filtrate become colorless.

Enzymatic Hydrolysis of Lemon grass leaf protein

Lemon grass total leaf protein (10 mg/mL) will be digested with chymotrypsin and trypsin (E/S = 1/100) for 5 h, 37 oC, andpH 7. 5 for the trypsin and pH 2. 0 for chymotrypsin, respectively. The reaction will be stopped by boiling for 10 min..

Protein Determination

The method of Bradford (year) will be used in determining the presence of proteins. Bovine serum albumin will be used as the protein standard and the absorbance at 280 nm will be measured.

Purification of Peptides

The tryptic and chymotryptic digests will be separated by RP-HPLC on an octadecyl silica (ODS) column (Cosmosil 5 C18-AR-II, 20×250 mm, Nacalai Tesque). The column will be eluted with a linear gradient of acetonitrile (1 %/min), containing 0. 1% trifluoroacetic acid (TFA) at a flowrate of 10 mL/min. The elution will be monitored at 230 nm and each peak will be collected as a separate fraction. All fractions will be dried with a centrifugal concentrator, and their ACE inhibitory activity will be measured.

Measurement of ACE-Inhibitory activity

Preparation of ACE

Rabbit lung acetone powder extract will be prepared by mixing fresh rabbit lung with acetone using a blender. The resulting mixture will be centrifuged at 7, 000 rpm for 30 minutes. Removal of acetone will be done via evaporation in the fume hood. The obtained powder will be dissolved in 100 mM phosphate buffer, pH 8. 3 (1: 10 w/v) and will be centrifuged at 4, 000 rpm at 4 oC for 75 min. The supernatant will be stored at -70 oC.

Spectrophotometric Assay of ACE activity

ACE activity determination will be patterned from the method of Cheung and Cushman (1979) along with some modifications. An assay mixture of 0. 25 mL containing 100 mM phosphate buffer, 300 mM sodium chloride, 5 mM hippuryl-L-histidine-L-leucine (HHL) and ACE from rabbit lung will be prepared. The ACE from rabbit lung will be added last to initiate the reaction. A 50 µL of the protein sample will be added in each assay mixture. The assay mixture will be incubated at 37 oC for 30 min. The enzymatic reaction will be completed by the addition of 0. 25 mL of 1 N HCl. For the control, 250 µL of 1 N HCl will be added first before the enzyme. The liberated hippuric acid will be extracted with 1. 5 mL ethyl acetate by vortex mixing for 15 s. One (1) mL of the ethyl acetate layer will be transferred to a watch glass and will be evaporated to dryness. Redissolution of the hippuric acid with 1. 0 mL water will be done and the resulting mixture will be subjected to spectrophotometric analysis at 228 nm for ACE activity determination.

The enzyme activity will be computed using the formula provided by Cushman and Cheung (1970). One (1) unit of angiotensin-converting enzyme

activity will be defined as the amount of catalyzing the formation of 1 µmole hippuric acid from HHL in 1 min at 37 oC under standard assay condition. The ACE activity, expressed in units (U), will be calculated using the formula:

- where A228 (blank) is the absorbance of the blank at 228 nm, A228 (control) is the absorbance of the control at 228 nm and 5. 6 x 10-3 is the conversion factor. The remaining angiotensin-converting enzyme activity will be computed using the equation below:
- where A228 (sample) is the absorbance of the sample at 228 nm. The inhibitory activity of the sample was determined by subtracting the remaining ACE activity from the ACE activity.

Computation of the percentage inhibition will be done using the equation from Zouari et. al (year?).

where A is the absorbance of hippuric acid generated in the presence of ACE inhibitor component (sample); B is the absorbance of hippuric acid generated without the ACE inhibitor (blank) and C is the absorbance of hippuric acid generated without ACE (control).

Characterization of ACE-Inhibitory Peptides

The ACE-Inhibitory activities of the tryptic and chymotryptic digests will be determined by evaluation of their IC50. Additionally, reaction mixtures will be analyzed using HPLC following preincubation with ACE. Samples will be injected on an ODS column (Cosmosil 5 C18-AR-II, 20×250 mm, Nacalai Tesque). The column will be eluted with a linear gradient if acetonitrile (0-40%, 1%/min.), containing 0. 1% TFA at a flow rate of 1mL/min.

A positive control will be also included in the experiment. Pulverized captopril which is known as an antihypertensive drug will be substituted to protein sample for ACE-inhibitory assay. IC50

The IC50 value is defined as the concentration of inhibitor required to inhibit 50% of the ACE activity under the assayed conditions and determined by regression analysis of ACE inhibition (%) vs log (inhibitor concentration in mg/mL).

Analysis of Protein by SDS-PAGE

Total Leaf proteins will be characterized by SDS-PAGE according to the method of Laemmli (year?). A 0. 75 mm separating gel in a final concentration of 11% and will be compared with pure commercially available rubisco (Sigma). The electrophoresis will be carried out using the Mini-PROTEAN II apparatus (Bio-Rad). Protein bands will be stained with Coomassie Brillant Blue R-250 in (state solvent),.

LITERATURE CITED

AFTAB, K., M. D. ALI, P. AIJAZ, N. BEENA, H. J. GULZAR, K. SHEIKH, Q. SOFIA, and S. TAHIR ABBAS. 2011. Determination of different trace and element in Lemon grass samples by x-ray fluorescence spectroscopy technique. International Food Research Journal, 18: 265-270.

BALASURIYA, B. W. N., and H. P. V. RUPASINGHE. 2011. Functional Foods in Health and Disease, 5: 172-188.

BRADFORD, MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. pp. 248-254.

BROWN, N., D. VAUGHAN. 1998. Angiotensin-coverting enzyme inhibitors. Journal of The American Heart Association.

CHAMPE, P. & R. HARVEY. Lippincott's Illustrated Reviews: Biochemistry. 1994. 2nd ed. J. B. Lippincott Company. Philadelphia. pp. 232-233.

CHATTERJEE, A. & A. BASU. 2011. Rubisco: Limitations and Re-engineering for a Better Enzyme. International Research Journal of Plant Science 2(2): 022-024.

CHEUNG, H., F. WANG, M. A. ONDETTI, E. F. SABO, and D. W. CUSHMAN. 1979. Binding of Peptide Substrates and Inhibitors of Angiotensin-Converting Enzyme. The Journal of Biological Chemistry, 255 (2): 401-407.

ERDMANN, K., B. W. Y. CHEUNG and H. SCHÓ¦DER. 2008. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. Journal of Nutritional Chemistry, 19: 643-654.

ERDÓ¦S, E. G., F. TAN, and R. A. SKIDGEL. 2010. Angiotensin I-Converting Enzyme Inhibitors Are Allosteric Enhancers of Kinin B1 and B2 Receptor Function. Journal of the American Heart Association.

GUANG, C., and R. D. PHILLIPS. 2009. Plant Food-Derived Angiotensin I-Converting Enzyme Inhibitory Peptides. Journal of Agricultural and Food Chemistry, 57: 5113-5120.

GUTTERIDGE, S. and A. A. GATENBY. 1995. Rubisco, Synthesis, Assembly, Mechanism and Regulation. American Society of Plant Physiologists, 7: 809-819.

HARTMANN, R., and H. MEISEL. 2007. Food-derived peptides with biological activity: from research to food applications. Current Opinion in Biotechnology, 18: 163-169.

HINDUMATHAY, C. K. 2011. In vitro Study of Antibacterial Activity of Cymbopogon citratus. World Academy of Science, Engineering and Technology, 74: 193-197.

KAMNERDPETCH, C. 2006. Enzymatic hydrolysis of peptide mapping of potato pulp protein. Ph. D. dissertation. University of Hannover. p. 114

KIM J. H., D. H. LEE, S. C. JEONG, K. S. CHUNG, and J. S. LEE. 2004. Characterization of Antihypertensive Angiotensin I-Converting Enzyme Inhibitor from Saccharomyces cerevisiae. Journal of Microbiology and Biotechnology, 14 (6): 1318-1323.

KORHONEN, H., and A. PIHLANTO. 2006. Bioactive peptides : Production and Functionality. International Dairy Journal, 16: 945-960.

KUBA, M., C. TAN, S. TAWATA, and M. YASUDA. 2005. Production of angiotensin I-converting enzyme inhibitory peptides from soybean protein with Monascus purpureus acid proteinase. Process Biochemistry, 40: 2191-2196.

KUBA, M., K. TANKA, S. TAWATA, Y. TAKEDA, and M. YASUDA. 2002. Angiotensin I- Converting Enzyme inhibitory peptides from tofuyo fermented soybean food. Biosci. Biotechnol, Biochem, 67: 1278-1283.

LEE, K. J., S. B. KIM, J. S. RYU, H. S. SHIN, and J. W. LIM. 2005. Separation and Purification of Angiotensin Converting Enzyme Inhibitory Peptides Derived From Goat's Milk casein Hydrosylates. Journal of Animal Science, 18(5): 741-746.

NAKANO, D., K. OGURA, M. MIYAKOSHI, F. ISHII, H. KAWANISHI, D. KURUMAZUKA, C. J. KWAK, K. IKEMURA, M. TAKAOKA, S. MORIGUCHI, T. IINO, A. KUSOMOTO, S. ASAMI, H. SHIBATA, Y. KISO, and Y. MATSUMURA. 2006. Antihypertensive Effect of Angiotensin I-Converting Enzyme Inhibitory Peptides from a Sesame Protein Hydrosylate in Spontaneously Hypertensive Rats. Biosci. Biotechnol. Biochem, 70: 1118-1126.

NWACHUKWU, I. N., L. N. ALLISON, E. C. CHINAKWE and P. NWADIARO. 2008. Studies on the effect Cymbopogon citratus, Ceiba pentardia and Loranthus bengwelensis extracts on species of dermatophytes. The Journal of American Science, 4(4).

OLOYEDE, O. I. 2009. Chemical profile and antimicrobial activity of Cymbopogon citratus leaves. Journal of Natural Products, 2: 98-103.

RHYU, M., Y. NAM, and H. LEE. 1996. Screening of angiotensin I – converting enzyme inhibitors in cereals and legumes. Foods and Biotechnology, 5 (4): 334-345.

ROY, HARRY. 1989. Rubisco Assembly: A Model System for the Studying the Mechanism of Chaperonin Action. American Society of Plant Physiologists, 1, 1035-1042.

YANG, Y. J., E. W. MARCZAK, M. YOKOO, H. USUL, and M. YOSHIKAWA. 2003. Isolation and Antihypertensive Effect of Angiotensin I-converting Enzyme (ACE) Inhibitory Peptides from Spinach Rubisco. The Journal of Agricultural and Food Chemistry, 51: 4897-4902.

YANG, Y. J., E. W. MARCZAK, H. USUL, Y. KAWAMURA and M. YOSHIKAWA. 2004. Antihypertensive Properties of Spinach Leaf Protein Digests. The Journal of Agricultural and Food Chemistry, 52: 2223-2225.

ZOUARI, S., N. ZOUARI, N. FAKHFAKH, A. BOUGATEF, M. A. AYADI and M. NEFFATI. 2010. Chemical composition and biological activities of a new essential oil chemotype of Tunisian Artemisia herba alba Asso. Journal of Medicinal Plants Research. 4(10): 871-880.