ACE (Angiotensin Converting Enzyme) Inhibition Activity of Oven – Dried and Air – Dried Sambong Blumea balsamifera L.(dc.) Tea

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ABSTRACT
ACE plays important roles by catalyzing the conversion of angiotensin I into the potent vasoconstrictor, angiotensin II. The latter inactivates vasodilator nonpeptide bradykinin and results in an increase of blood pressure. Moreover, inhibition of ACE has proven to be an effective strategy in prevention and treatment of hypertension. Clinically, synthetic ACE inhibitors such as captoril, enalapril, are used for hypertension. However, these agents exert pronounced side effects such as cough, angioedema and many more. A major challenge is to search new drugs that will be more selective and thus, have lesser side effects. Sambong, Blumea balsamifera, is abundant in the Philippines and recommended by the Department of Health as a tea preparation for the treatment of kidney stones. Up to date, no published studies yet on the ACE inhibitory activity of Sambong. The study delved into the angiotensin converting enzyme inhibition activity of Sambong tea. Specifically, the absorbance reading of FAPGG (Furylacryloyl – Phenylalanine – Glycyl – Glycine) at 340 nm after exposition to the oven - dried and air – dried Blumea balsamifera tea and its inhibitory activity. This study employed rabbit lung ACE – induced hydrolysis of FAPGG quantified using a spectrophotometer. Quality control tests were performed on the tea samples. Study revealed that Sambong tea preparation showed inhibitory activity on rabbit lung ACE. It has been observed that the air – dried tea has a mean inhibitory activity of 211.30% while the oven – dried tea has a mean inhibitory activity of 70.42%. Flavonoids and terpenoids were attributed to the inhibition of ACE.

Keywords: angiotensin converting enzyme inhibitor; Blumea balsamifera; tea.

INTRODUCTION
CVDs (Cardiovascular diseases) are the number one cause of death globally; more people die annually from CVDs than from any other cause. Over 80% of the world's deaths from CVDs occur in low- and middle-income countries which makes it a due concern for a country such as Philippines. Each year, 9.4 million or 16.5% of all deaths can be attributed to high blood pressure4. In the Philippines, diseases of the heart, like hypertension and myocardial infarction, are among the top three leading cause of morbidity and mortality2. Hypertension is defined by the presence of a chronic elevation of systemic arterial pressure above a certain threshold value. It is a progressive cardiovascular syndrome arising from complex and interrelated etiologies. Pharmacologic therapy with one or more drugs is warranted when lifestyle modification fails to prevent or correct hypertension. Diuretics, angiotensin receptor blockers, beta blockers, and ACE inhibitors are some of the drug used in the treatment and management of hypertension3. The influences of ACE on blood pressure make it an ideal target clinically and nutritionally in the treatment of hypertension. Inhibition of ACE mainly results in an overall antihypertensive effect4,5. Angiotensin I converting enzyme (ACE or kinase 11; E.C 3.4.15.1) is a dipeptidyl – carboxypeptidase containing zinc ion in its active site. ACE plays important roles in rennin – angiotensin and kallikrein – kinin systems by catalyzing the conversion of the inactive decapeptide, angiotensin I into the potent vasoconstrictor, octapeptide angiotensin II. Furthermore, it inactivates vasodilator nonapeptide bradykinin and results in an increase of blood pressure. Inhibition of ACE has proven to be an effective strategy in prevention and treatment of hypertension and related diseases4. Synthetic ACE inhibitors such as captoril, enalapril, lisinopril or temocapril are in clinical use for hypertension. However, as those ACE inhibitors exert well described side effects such as allergic reactions, skin rashes, cough, taste disturbances, drug – drug interaction, there is a constant interest in discovering novel compounds with ACE inhibitor potential as alternatives to synthetic drugs4. A major challenge is to search and design new drugs that will be more selective in inhibiting ACE, and thus have lesser side effects. Sambong, Blumea balsamifera, its crude extract and isolated constituents display numerous biological activities, such as superoxide radical scavenging, antioxidant, antimicrobial and anti – inflammation, anti – plasmodial, anti – tyrosinase, platelet aggregation, enhancing percutaneous penetration, wound healing, and many more. Phytochemicals present in Sambong include flavonoids, terpenes (monoterpenes, sesquiterpenes, diterpenes), organic acids, esters, and sterols. Flavonoids in food were reported to be potent
inhibitors of ACE. In the Philippines Sambong is approved by the Department of Health as a tea preparation for the treatment of kidney stones. Drying techniques employed in the production of tea includes air – drying or oven – drying. These techniques, however, have not been evaluated as to its effects to its constituents and its purported activity. Up to date, no published studies yet on its ACE inhibitory activity of Sambong. Thus, the ACE inhibitory activity of the oven – dried and air – dried Blumea balsamifera tea was conducted.

MATERIALS AND METHODS

Preparation and Production Blumea balsamifera Tea

Leaves of Blumea balsamifera were supplied by the Philippine Institute of Traditional and Alternative Health Care (PITAHC) - Tacloban. Leaves were carefully selected utilizing only fresh, mature and injury – free leaves. The leaves were thoroughly washed with tap water to remove dirt and other impurities. Five hundred grams of the leaves were air – dried while the other five hundred grams were oven - dried at 40 °C until it was dry to touch. Moisture content of the oven – dried and air – dried leaves was monitored using a moisture analyzer. The leaves were then milled separately using a Wiley mill. Three grams of milled oven – dried and air – dried B. balsamifera leaves were separately placed in individual pre - fabricated tea bags. A sample of the tea was deposited at the University of San Carlos – Department of Pharmacy Research Laboratory.

Quality Control Testing of Blumea balsamifera Tea

For the quality control testing, prepared tea powder underwent organoleptic evaluation, microbial analysis for fecal coliforms and total coliforms, and moisture content determination using a moisture analyzer.

Preparation of Tea Test Solutions

Three grams of milled air - dried Blumea balsamifera leaves were placed in individual pre - fabricated tea bags. Each tea bag was infused with 180 mL of 100 °C distilled water for five minutes. The resulting tea was then labelled air – dried tea. Moreover, similar amount and process were done for the oven – dried Blumea balsamifera leaves and the resulting tea was then labelled oven – dried tea.

Preparation of Positive Control

A tablet containing 25 mg of Captopril was pulverized using a mortar and pestle. The pulverized drug was mixed with sufficient amount of water to reach 25 milliliters obtaining a concentration of 1 mg/mL.

Preparation of Substrate and Buffer

Tris – HCl (0.2M) was used as the buffer in the preparation of the substrate, FAPGG. The Tris - HCl was prepared by dissolving 1.21 grams of Tris in sufficient amount of water to reach 50 mL. The resulting solution was added with 21.9 mL of 0.2M HCl to obtain a pH of 8.2 and diluted with water to reach a volume of 200 mL. FAPGG (0.5mM) (Sigma – Aldrich, Singapore) was dissolved in sufficient amount of Tris – HCl to reach a volume of 50 mL.

Preparation of ACE Enzyme

ACE from rabbit lung 25 mg (EC 3.4.15.1, Sigma – Aldrich, Singapore) was used as the enzyme for the assay. The enzyme was made into a solution by the addition of sufficient amount of buffer to reach 25 mL having a concentration of 1 mg/mL.

ACE Inhibition Assay

Over – dried tea and air – dried tea were tested for ACE inhibitory activity using FAPGG as the synthetic substrate for ACE and using the method of Raghavan and Kristinsson (2009) and Shori and Chuah (2013). Tea samples (100 µL), ACE (50 µL) and FAPGG, substrate (1 mL), were mixed in a test tube and incubated for 60 minutes at 37°C. At the end of 60 minutes incubation period, the solution was transferred to a cuvette and diluted with distilled water to reach brim of the cuvette then absorbance of the samples were measured at 340 nm using an UV – Vis spectrophotometer. Hydrolysis of FAPGG by ACE would result in a decrease in absorbance at 340 nm. ACE inhibitory activity was measured by the ability of the tea to decrease hydrolysis of FAPGG. The same procedure was done prior to the determination of the absorbance of blank and positive control. A sample containing ACE (50 µL) and FAPGG (1 mL) was used as the blank while captopril (100 µL) was used as the positive control. ACE inhibitory activity was computed using the formula below.

Percent ACE Inhibitory Activity =

(Abs sample – Abs blank) * 100
(Abs positive – Abs blank)

RESULTS AND DISCUSSION

Description of Powder and Tea

The oven – dried powder was brown in color with cotton – like mass while the air – dried powder was green in color with cotton – like mass. Both powders feel soft when touched and coarse when rubbed. Moreover, the oven – dried tea and air - dried tea were clear and free from particles however, the air – dried tea appeared to be darker brown than the oven – dried tea.

ACE Inhibition Assay

Figure 1 shows the mean absorbance at 340 nm of the tea, positive control and the blank per trial. Among the four trials, the air - dried tea showed the highest absorbance reading nearly 1.4, followed by, the oven – dried tea with an absorbance reading of nearly 0.8 for all trials. Lastly, the blank revealed the lowest absorbance reading around 0.54. Figure 2 reveals the percent inhibitory activity of the oven – dried tea and the air – dried tea on ACE. It has been observed that the air – dried tea has a mean inhibitory activity of 211.30% while the oven – dried tea has a mean inhibitory activity of 70.42%. The inhibitory activity of the air – dried tea is thrice higher compared to that of the oven – dried tea. Table 1 presents the quality control test results conducted in this study. Blumea balsamifera tea prepared by oven – dried and air – dried inhibited angiotensin converting enzyme. Tea prepared by air drying exhibited higher inhibition activity compared to tea prepared by oven – drying. ACE has two active sites, N – terminal and C – terminal, having different affinities for substrates. Inhibition of ACE arises from competitive binding with substrate on the active sites. The substrate (FAPGG) is acted upon by the ACE and it is converted into FAP and GG. FAPGG is a tripeptide blocked at the N-terminal by a furanacryloyl group, which acts as a chromophoric
The absorbance of FAPGG is observed under a UV–VIS spectrophotometer at 340 nm. The relationship between absorbance and inhibition activity is directly correlated. An increase in the absorbance reading of the substrate indicates greater inhibition of ACE. It was noted that the tea prepared by air drying has higher absorbance compared to the oven–dried tea. This would indicate that tea prepared by air drying was able to inhibit more of the ACE compared to oven–dried tea, preventing it from acting on the FAPGG and consequently greater absorbance reading of the substrate at 340 nm. ACE critically regulates the renin–
angiotensin–aldosterone system and plays a major role in the control of blood pressure. Increased ACE activity promotes hypertension because this enzyme promotes vasoconstriction by converting angiotensin I into the powerful vasoconstrictor angiotensin II, and by inactivating the vasodilator bradykinin. Inhibition of ACE will prevent the conversion of angiotensin I to angiotensin II, thereby preventing vasoconstriction of blood vessels and important in the control of blood pressure. Several studies on phytochemical screening of Sambong revealed the presence of flavanoids and terpenes. In this study, both forms of tea contain flavanoids and terpenes. These phytochemicals are parallel with several works claiming that flavanoids and terpenes are potent inhibitors of ACE. Sambong tea found to be containing terpenes is a good candidate for the management of hypertension as it has been shown to inhibit enzyme important in the regulation of the rennin – angiotensin system. On the other hand, as ACE is a membrane – bound enzyme, flavanoids can adsorb to lipid – water interphases, it is feasible that a local enrichment of flavanoids on membrane surface could lead to an enhanced interaction with the enzyme. This accumulation could take place on the membrane of vascular endothelial cells, which are thought to be responsible for regulating blood pressure. Among flavonoids, flavan-3-ols and anthocyanins are effective ACE inhibitors in vitro as well as in animal model system. Catechins and their polymers proved to be the most effective ACE inhibitor in vitro. However, the results of the in vitro studies may not reflect exactly the outcome of in vivo studies. Therefore, further studies using animal models are required to confirm their ACE inhibitory properties. Isoflavones are showing intermediary inhibition towards ACE. Flavonols had proved to be less effective in vitro but in animal studies they were found to be more effective. Fewer studies had been conducted on flavones and chalcones. Structurally modified flavonoids designed for greater absorption and bioavailability could have a higher potential in use as ACE inhibitors. In terms of the mode of action, flavonoids had shown competitive type inhibition for ACE. Blumea balsamifera tea prepared by oven – drying and air – drying exhibited inhibitory activity of angiotensin - converting enzyme. The air – dried tea possessed thrice higher inhibitory activity compared to the oven – dried tea. Blumea balsamifera tea is best prepared by air – drying than oven – drying as it showed higher inhibition activity, while for oven drying; some constituents might be heat sensitive and leads to lesser inhibition activity. Terpenoids and flavanoids were found in both preparations that are attributed to the inhibition of angiotensin converting enzyme. Isolation of terpenoids and flavanoids from the Blumea balsamifera tea must be conducted and quantified. Further testing such as clinical testing must be done on the tea to further establish safety and effectiveness of the product.

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