CHAPTER 2

LITERATURE REVIEW

2.1   Hypertension

Hypertension or high blood pressure is a worldwide problem that effects approximately 15-20% of all adults (Wang et al., 2008). Hypertension known as silent killer as it showed no symptom. Even though it is simple to diagnose and usually can be controlled by healthy diet, regular exercise, medication prescribed by doctors or a combination of these, untreated hypertension will cause serious condition (Campbell et al., 2002). Hypertension is associated with cardiovascular disease, insulin resistance, obesity, carbohydrate tolerance, hyperuricacidemia, and atherosclerosis (Yeh et al., 2009). Hypertension affects the structures and functions of small muscular arteries, arterioles and other blood vessels and can cause damage at variable rate to various target organs including kidney, brain and eye, related with the end stage of renal disease and to be the cause of stroke (Hock et al., 1995; Lee et al., 2010; Escobales et al., 2005). It is associated with the alterations in the blood vessels wall that affecting the endothelium, the media and the adventitia, whereas alteration in the media leading to remodeling of the vessel wall (Escobales et al., 2005). Patients with hypertension die prematurely with the most common cause of death are heart disease, while strokes and renal failure are frequently occurring, particularly in those with significant retinopathy (Jinglun et al., 1995).

Various antihypertensive drugs such as beta-blocking agents, hypotensive diuretics, calcium antagonist, angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor antagonists and alpha-receptor blocking agents were usually used to control hypertension and its alleviate symptoms clinically. Two or more

antihypertensive drugs from different categories usually were combined to achieve optimal results as the efficacy of these drugs is only about 40-60% (Yeh et al., 2009).

2.1.1 Essential hypertension

95% of all hypertension cases were categorized as essential hypertension that also known as primary hypertension or idiopathic hypertension (Carretero et al., 2000). It is a heterogeneous disorder as different patients have different factors that cause high blood pressure (Carretero et al., 2000). The cause of essential hypertension is still unknown but it is considered as the sum of interaction between genetic and multiple environmental factors (Büssemaker et al., 2010). Environmental factors including obesity, high alcohol intake, high salt intake, insulin resistance, low potassium intake, aging, sedentary lifestyles, stress, and low calcium intake contribute to the development of hypertension (Carretero et al., 2000). Inherited blood pressure (Bp) known as blood pressure that are genetically determined, while hypertensinogenic factors are factors that cause high blood pressure such as obesity, high alcohol and salt intake (Carretero et al., 2000).

Various of gene might involve in the development of hypertension can cause inherited blood pressure and the influences of these genes have been demonstrated by family studies that showed high blood pressure are associated among siblings and between parents and children (Carretero et al., 2000). Obesity is known as important risk factor for type 2 diabetes and cardiovascular disease (CVD) (He et al., 2009). It is associated with an incidence of arterial hypertension and known to be one of powerful risk factors for non-communicable diseases (Florencio et al., 2004). Obesity also acknowledge as the main hypertensinogenic factor compared to high alcohol intake, high salt intake, stress, sedentary lifestyles, dyslipidemia, low potassium and low

calcium intake (Carretero et al., 2000). According to the study in Shanghai on Chinese adults age 40 years and above, subject with obesity are significantly has higher risk of hypertension and type 2 diabetes (He et al., 2009). Obesity can cause insulin resistance, adult-onset diabetes mellitus, left ventricular hypertrophy, hyperlipidemia and atherosclerotic disease (Carretero et al., 2000). However, the mechanism of obesity raises blood pressure (Bp) is not fully understood (Carretero et al., 2000)

2.1.2 Secondary hypertension

Secondary hypertension can be caused by medical conditions such as renal parenchymal disease, renal artery stenosis, hyperaldosteronism, or pheochromocytoma (Grossman et al., 2012). Temporary high blood pressure also can cause by medications such as corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), cold medicines and birth control pills. Corticosteroids such as prednisone and prednisolone will lead to Cushing syndrome in long-term use. Usage of nonsteroidal anti-inflammatory drugs (NSAIDs) increase blood pressure as well as will interfere in anti-hypertensive treatment, and abolish its effect (Grossman et al., 2012). NSAIDs interfere in some of the antihypertensive agents such as beta-blockers, diuretics agents as well as angiotensin converting enzyme inhibitors (ACEI), except for calcium antagonist and central-acting drugs (Grossman et al., 2012). NSAIDS such as indomethacin, naproxen and piroxicam were the greatest that involves in the increasing of blood pressure, while rofecoxib raise systolic blood pressure more than celecoxib (Grossman et al., 2012).

Cold medicines such as pseudoephedrine hydrochloride that used for upper respiratory decongestant may elevate blood pressure in hypertensive patients (Grossman et al., 2012). Intake of birth control pills contributes in the increasing of blood pressure particularly in women above 35 years old that overweight and smokers.

Treatment for primary and secondary hypertension were specifically different thus, it is important in terms of its diagnostic, therapeutic and prognostic to be determined before patient with such conditions were treated (Seeman et al., 2005).
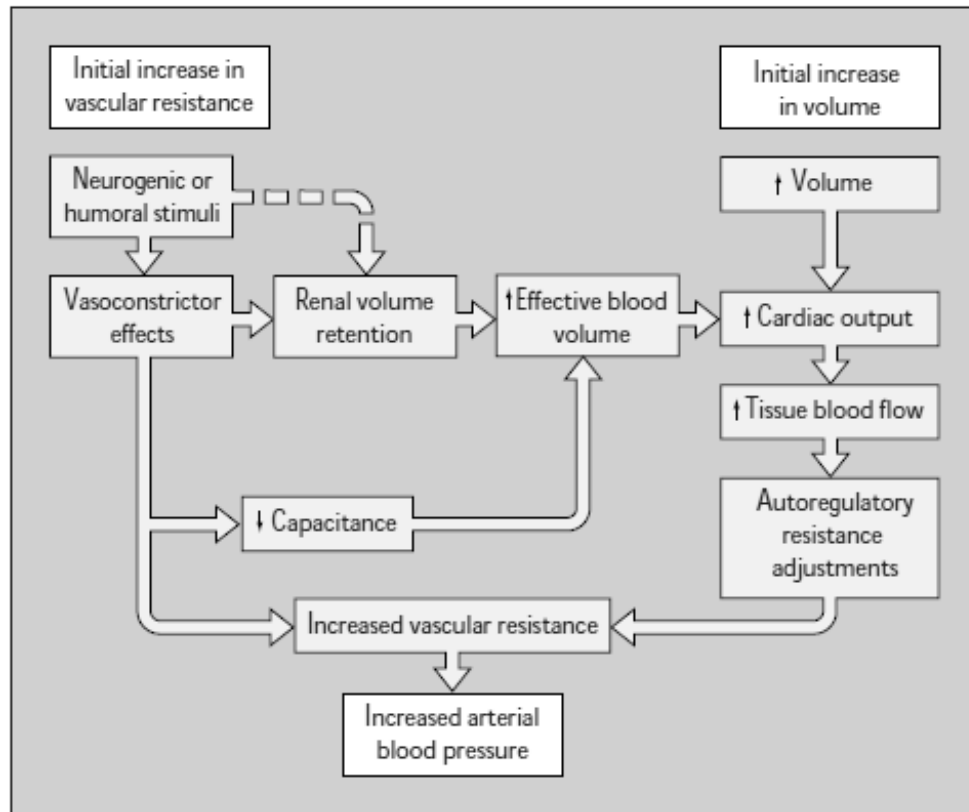

Figure 2.00. The mechanism mediating hypertension (Navar et al., 1997)

Increasing of arterial blood pressure can be caused by several factors such as increased in vascular resistance and initial increase in volume. Neurogenic and humoral stimuli stimulates vasoconstriction of blood vessel and cause renal volume retention that lead to increasing of cardiac output, tissue blood flow and vascular resistance that has cause increasing of arterial blood pressure. Increasing of blood volume also lead to vascular resistance thus induced blood pressure (Figure 2.00) (Navar et al., 1997).

2.2   Renin Angiotensin Aldosterone System (RAAS)

Renin Angiotensin Aldosterone System (RAAS) is part of a complex feedback circuit that play an important role in maintaining blood pressure homeostasis, fluid and salt balance in mammals (Campbell et al., 2002; Lee et al., 2010). It is considered as hormonal regulator of blood pressure together with sodium, potassium and water balance as it involves in both short-term and long-term regulation of blood pressure (Sharifi et al., 2004, Starr et al., 1994). The increasing activity of RAAS hormonal system has been associated with the development and maintaining of hypertension thus increase the interest in pharmacologic treatment for hypertension that involved Renin angiotensin aldosterone system (RAAS) (Igic et al., 2007; Starr et al., 1994). The malfunction of RAAS may affects functions of the heart, blood vessels and kidneys as it is an important regulator of blood pressure as well as electrolyte balance (Igic et al., 2007).
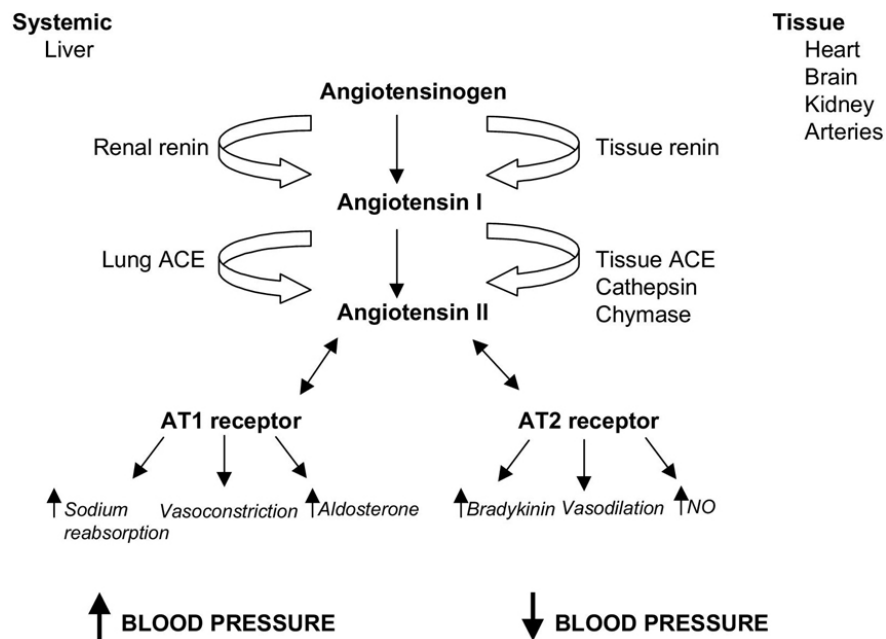
Figure 2.01. Renin-Angiotensin-Aldosterone System (RAAS) role regulation of blood pressure. ACE= Angiotensin Converting Enzyme; AT1= Angiotensin II Type 1; AT2= Angiotensin II Type 2; NO= Nitric (Schmieder, 2005)

In the regulatory system, kidney secretes renin that acts on a protein (angiotensinogen) in the bloodstream to form angiotensin I, a relatively inactive substance that can be converted into a most potent constrictor of blood vessels, angiotensin II (Hock et al., 1995). Angiotensin II (angiotensin II-octapeptide) is formed after the removal of dipeptide His–Leu from Angiotensin I in the lung by the action of kinase II, an angiotensin-converting enzyme (ACE). Angiotensin II is then transported in the circulation to various effectors target sites which are blood vessels, kidney, and adrenal gland, to interact with specific receptors to exert its action, thus play an important role in aldosteronogenesis. Angiotensin II interacts with the angiotensin 1 (AT1) receptors to increase the synthesis and release of aldosterone that in turn will acts mainly on the nephron proximal tubules to excretes the potassium and hydrogen into the tubule and increase the sodium reabsorption (Figure 2.01) (Skotnicka, 2003).

2.3 Angiotensin Converting Enzyme (ACE)

Angiotensin I Converting Enzyme (ACE) belongs to the class of zinc proteases that activated with the presence of zinc and chloride (Lee et al., 2010). ACE gene in human is located on chromosome 17 and it consists of 25 exons (Igic et al., 2007). The role of ACE in cardiovascular pathologies such as cardiac hypertrophy and hypertension as well as in renal disease was well establish (Dive et al., 2009;Dzau et al., 2001). It plays an important physiological role in regulation of blood pressure by virtue of renin angiotensin system by catalyst the conversion of angiotensin I to a potent vasoconstrictor, angiotensin II and inactivates a potent vasodilator, bradykinin (Lee et al., 2010; Lahogue et al., 2010; Sharifi et al., 2005). ACE enzyme presence in a two different molecular weight; a high molecular weight ACE enzyme was found in endothelial, epithelial and neuronal cells while low molecular weight ACE enzyme was

located in germinal cells (Dzau et al., 2001). ACE located in the endothelial cells can be found throughout the body while ACE located in epithelial cells can be found in gut and kidney (Meng et al., 1995). It exists as a membrane-bound enzyme in kidney, heart, and blood vessels, as well as exist in a freely soluble form in plasma (Sharifi et al., 2005). It can be detected in body fluid such as the blood, lymph, urine, cerebrospinal fluid and aqueous humor (Igic et al., 2007). ACE enzyme also are rich in blood vessel of the lung, retina, brain and kidney (Igic et al., 2007).

Inhibition of ACE enzyme is considered as therapeutic strategy and play an important role in controlling hypertension as a number of drugs has developed to inhibit enzymatic activity of ACE and were proved to reduce blood pressure successfully in hypertensive patients (Lee et al., 2010). ACE become an excellent target for therapeutic approach in the treatment of cardiovascular disease as its mechanisms of action is in the biosynthesis of angiotensin II (Dive et al., 2009).

2.3.1 Angiotensin Converting Enzyme (ACE) mechanism

Blood pressure regulation via two different reaction involving ACE enzyme that converts angiotensin I that also known as inactive peptide, to a powerful vasoconstrictor Angiotensin II and inactivate the synthesis of vasodilator peptide, bradykinin (Marczak et al., 2003). The conversion of angiotensin I to angiotensin II causes constriction of blood vessels that lead to increasing of blood pressure. Angiotensin II stimulates the secretion of hormones aldosterone from the adrenal cortex that causes the increasing of sodium and water reabsorption at kidney thus increase the volume of body fluid. Therefore, the blood pressure was induced via sodium retention (Oh et al., 2002). The expression of ACE in cultured endothelial cells was modulated by

steroids, calcium ionophores and growth factors while its level increased after confluence (Dzau et al., 2001).
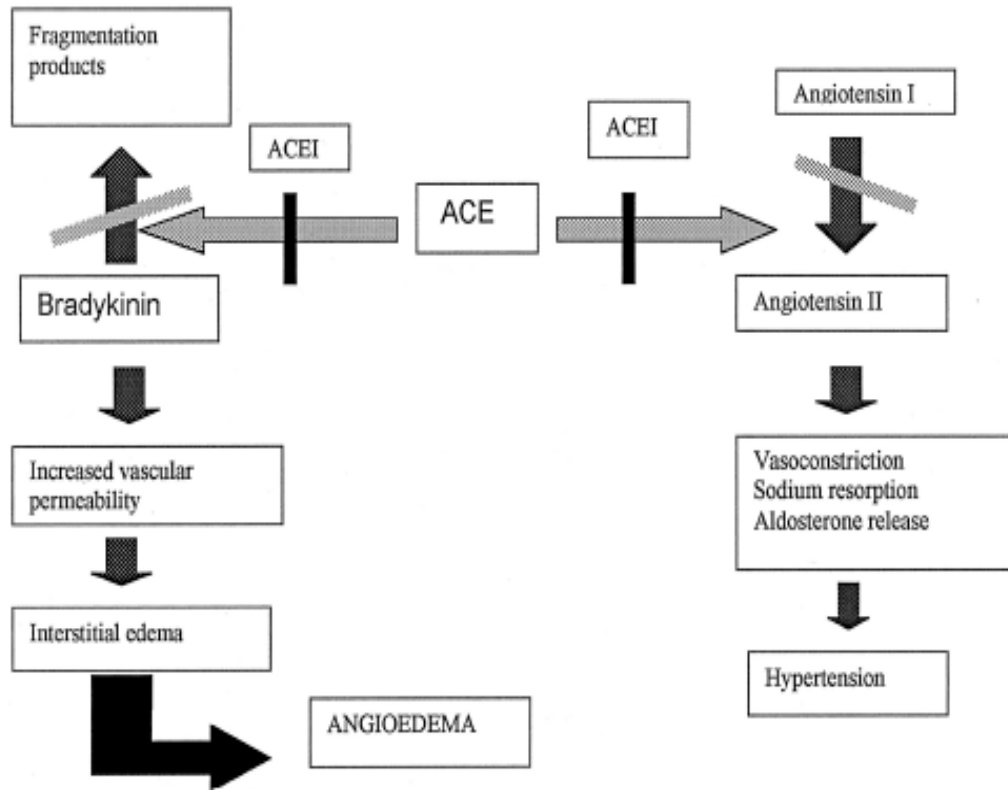

Figure 2.02. Mechanism of Angiotensin Converting Enzyme (ACE) in hypertension and angioedema (Sondhi et al., 2004).

From Figure 2.02, formation of Angiotensin II from Angiotensin I was inhibited by Angiotensin Converting Enzyme Inhibitor (ACEI), thus inhibit the mechanism of hypertension, while ACEI also stimulate secretion of bradykinin that cause increasing in vascular permeability and lead to angioedema (Sondhi et al., 2004). The absent of ACE inhibitor can cause formation of angiotensin II, a potent vasoconstrictor that will cause vasoconstriction of blood vessel, stimulates aldosterone and increased sodium retention thus induced high blood pressure.

2.3.2   Angiotensin Converting Enzyme inhibitors (ACEI)

Angiotensin converting enzyme (ACE) inhibitors constitute an establish therapy in the treatment of hypertension (Nyman et al., 1998). It principle action has targeted tissue site of ACE in its mechanism of action to reduce high blood pressure effectively as well as it possess exert cardio and renoprotective actions (Dzau et al., 2001). ACE inhibitors prevent the formation of angiotensin II that were identified as the factor that increase blood pressure. Therefore, the inhibition of the formation of angiotensin II leads in decreasing of blood pressure (Otte et al., 2007). The inhibition of ACE activity also cause in the buildup of bradykinin that will accumulate and will be responsible for the side effects that associated with ACE inhibition (Dive et al., 2009). Both the angiotensin II and bradykinin were responsible in vascular tone. Whereas ANG II acts as vasoconstrictor and growth promoting substance, bradykinin act as antagonist of ANG II and react as vasodilator and growth inhibitor (Tom et al., 2003).

ACE inhibitors prevent the progression of heart and kidney disease with the interruption in the renin-angiotensin system at different stages including prevent degradation of bradykinin (Thurman et al., 2003; Tom et al., 2003). Increasing level of bradykinin also inhibit ACE enzyme thus responsible in the natriuresis, extracellular matrix degradation, angioedema as well as vasodilation of vascular tone (Figure 2.03) (Thurman et al., 2003). However, non-ACE pathways or "angiotensin escape" happen as level of angiotensin I increased, thus responsible in the angiotensin II formation. At this stage, it is AT_1 blockers responsibility to block angiotensin II at AT_1 receptor and make it available for AT_2 receptor to stimulate and triggers the bradykinin or nitric oxide (NO) or cyclic guanosine monophosphate cascade (Thurnan et al., 2003).

Clinical studies found that ACE inhibitors have a protective effect on ventricular functions after myocardial infarction, improve cognition and protect renal. The significant role of ACE peptide inhibitors was recognized as the treatment of hypertension when it was first discovered from snake venom (Wu et al., 2002). During research programmed in the 1960s and 1970s, a number of different ACE inhibitors were identified and Captopril was the first commercially available product (Starr et al., 1994). It is marketed under the name Capoten by Bristol-Myers Squibb. It inhibits the action of angiotensin converting enzyme (ACE), thus vasolate blood vessel and help lowering blood pressure. Captopril and Enalapril are the synthetic ACE inhibitors that are widely used to treat hypertension and some types of congestive heart failure (Oh et al., 2002). It is also proved that it have significant vasculoprotective and cardioprotective affect, reduce stress and reduce inflammation in the endothelium, improves blood flow as well as flow-mediated vasodilation (Berra et al., 2009). Both ACE inhibitors inhibit the progression of diabetic nephropathy and chronic renal failure (Bartosz et al., 1997).

Clinical data have shown that Captopril and other ACE inhibitors has not only reduce high blood pressure in essential hypertension but also reduce cardiovascular risk via bradykinin accumulation in biological fluids as well as blocking the RAS circulation (Igic et al., 2007). ACE inhibitors prevent the binding and cleaving of its major substrate, angiotensin I and bradykinin by binding itself to the active site of ACE (Igic et al., 2007). ACE inhibitors were categorized via its structure into three groups which are compound contain sulfhydryl, phosphinyl or carboxyl side groups (Igic et al., 2007).
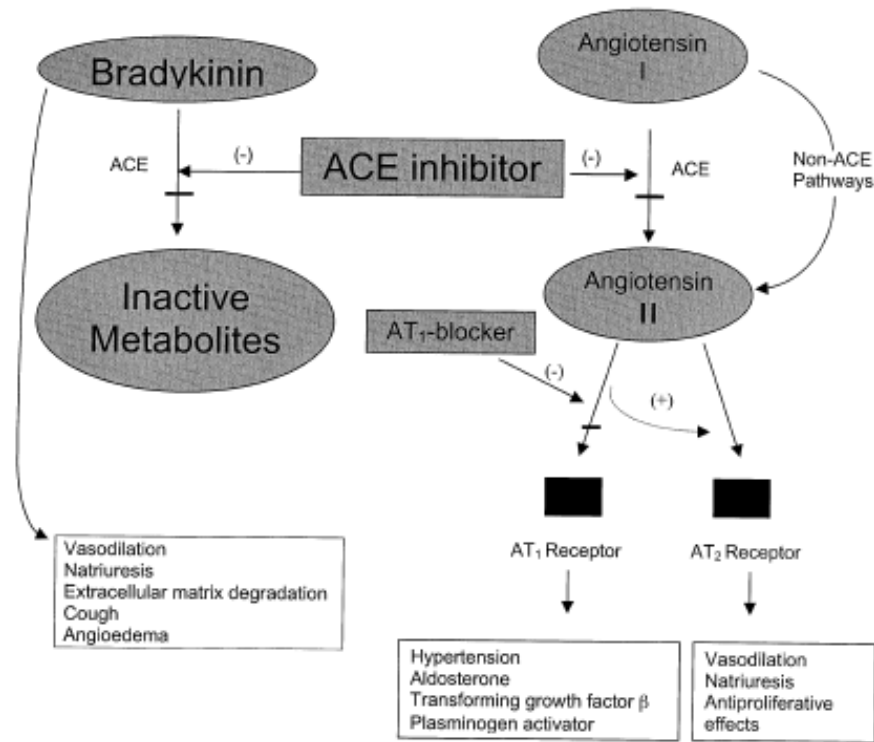
Figure 2.03. Role of ACE inhibitors and angiotensin II type 1 (AT1) receptor blockers. ACE inhibitors inhibit the formation of angiotensin II by ACE and block kinase activity of ACE to increase kinins's level (Thurman et al., 2003).

Captopril ([2S]-1-[3-mercapto-2-methyl-propionyl]-L-proline) (Figure 2.04(a)) was the first ACE inhibitors found and has important role in reducing hypertension. It protects erythrocytes from hemolysis caused by 2,2'-azobis (2-amidinopropane) (AAPH) and hypochlorite, inhibited ascorbate autoxidation caused by Cuprum (Cu^{2+}) that indicates ability of Captopril as antioxidant (Bartosz et al., 1997). Together with Enalapril (Figure 2.04(b)), both ACE inhibitors were equally active to avert oxidation of Low Density Lipoprotein (LDL) and increase activities of superoxide dismutase and glutathione peroxidase in experimental animal's liver, while activities of erythrocyte Na^+, K^+-ATPase and Ca^{2+}-ATPase in hypertensive patients is increase as well (Bartosz et al., 1997). Compare to Enalapril, Captopril has advantages as it has the ability to prevent arrhythmias caused by ischemia or reperfusion, to protect cultured

endothelial cells against free radical injury as well as anti-inflammatory activity (Bartosz et al., 1997).

However, Captopril treatment bring unwanted side effects such as renovascular disease, cough, neutropenia, skin rashes, angioneurotic edema and taste disturbance that cause by the sulfhydryl moiety (Choi et al., 2001). It is also has a poor pharmacokinetic profile and need to be consumed 2 to 3 times daily due to short half-life. Pharmacokinetics limitations and unwanted side effect of Captopril bring the development of enalapril and other subsequent ACE inhibitors that were specifically design to avoid rashes and taste disturbance by elimination of the sulfhydryl moiety. Its half-life was pro-long to improve patient compliance.
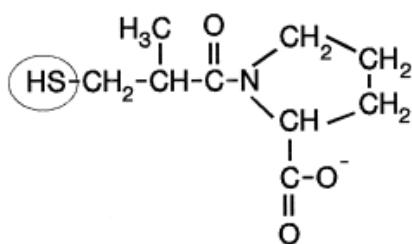
a)   Captopril
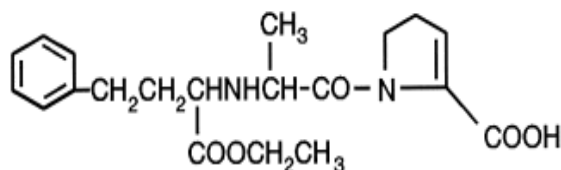
b)   Enalapril

Figure 2.04. Chemical structures of ACE inhibitors (Bartosz et al., 1997).

Nutrition is one of the main factors that influence the blood pressure (Lahogue et al., 2010). It is easy and effective in controlling blood pressure without any excessive decrease, thus the physiologically functional foods might be the best candidates in the nutritional approach (Yoshii et al., 2001). Study have been focused on the identification of food components that have the potential to inhibit ACE activity to control hypertension thus to prevent cardiovascular disease through diet (Lahogue et al., 2010). Various natural ACE inhibitors from functional food and natural bio-resources have been isolated as the researchers has great interest in finding the non-toxic ACE inhibitors as an alternatives to synthetic drugs (Lee et al., 2010). ACE inhibitors also promotes the release of nitric oxide, vasodilation and natriuresis as well as prevent the degradation of vasoactive peptide bradykinin (Berra et al., 2009).

Identification and isolation of bioactive compounds with anti-ACE properties were assessed using several reliable methods to measure ACE activity such as spectrophotometry, bioassay, fluorometry, High Performance Liquid Chromatography (HPLC) and capillary electrophoresis (Lahogue et al., 2010).

This study was based on the modification protocol from Lahogue et al., (2010) that also a method preferred by the pharmaceutical and food industries (Lahogue et al., 2010). It is based on the hydrolysis of a synthetic peptides hippuryl-histidyl-leucine (HHL) by ACE to give Hippuric acid and histidyl-leucine products. The amount of Hippuric acid form from HHL by action of ACE is extracted using ethyl acetate and the concentration was determined by spectrophotometric assay at 228 nm (Wu et al., 2002, Lahogue et al., 2010). Animal study was carried out in the determination of anti-hypertension effects of water extracts of *Tacca integrifolia* on male Spontaneously Hypertensive Rats (SHR).

2.4   **Antioxidants**

Exposure to a polluted environment cause by pesticides, toxic chemical waste, direct and second hand cigarette smoke, gasoline exhaust, air pollutants and radiation as well as physical stress has cause a toxic effects on human health as these environments produce high amounts of free radicals, hence contribute to human disease pathophysiology (Bagchi et al.,2000). Free radicals produce in polluted environment will lead to lipid, proteins and DNA deterioration, activation of procarcinogens, inhibit cellular and antioxidant defense system as well as depletion of sulfhydryls, altered calcium homeostasis, induction of abnormal protein and changes in gene expression (Bagchi et al.,2000).

Oxidation reactions that produce free radicals start the chain reaction in damaging cells while a molecule call antioxidant that capable in slowing or preventing the oxidation, donate one of its own electrons to end the electron stealing reaction hence neutralize the free radicals as in Figure 2.05 (Talaulikar et al., 2011). Antioxidant can be classified as antioxidant vitamins, antioxidant minerals and antioxidant enzymes and it has been studied extensively to prevent various degenerative diseases including carcinogenesis (Bagchi et al., 2000). Antioxidant vitamins such as vitamin C and vitamin E, β-carotene and proanthocyanidins, antioxidant minerals such as zinc and selenium while glutathione, superoxide dismutase and catalase were classified under antioxidant enzymes (Bagchi et al., 2000). Table 2.00 showed several chemical structure of several antioxidants including polyphenol and flavonoid. Inhibition of antioxidant enzyme or low level of antioxidants can cause oxidative stress and may lead to cell damage (Talaulikar et al., 2011).
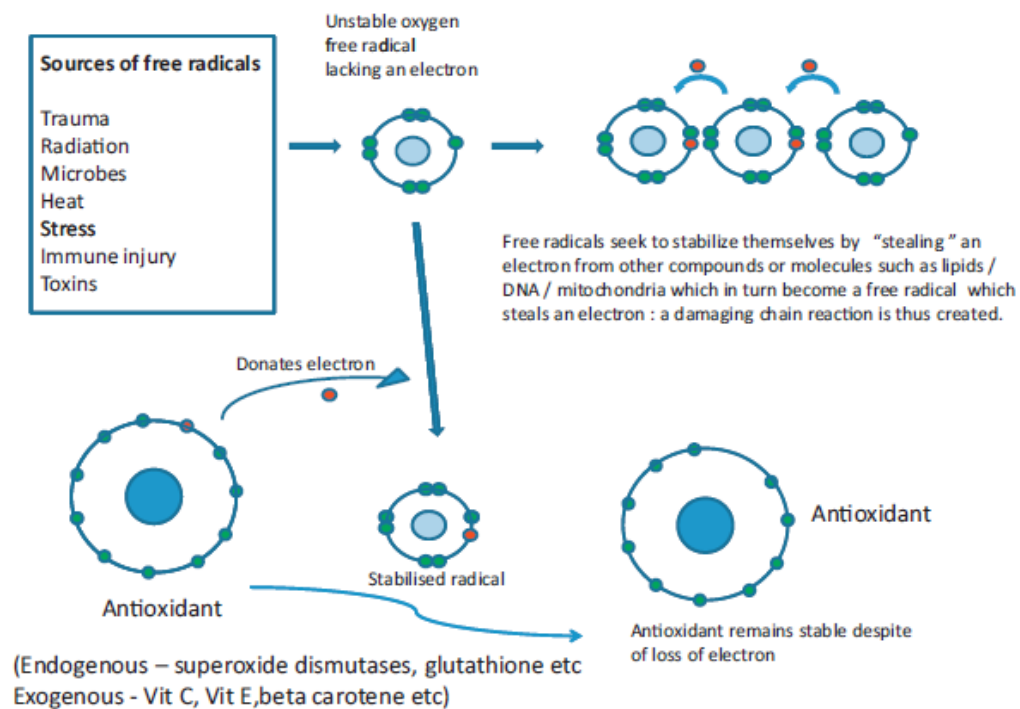
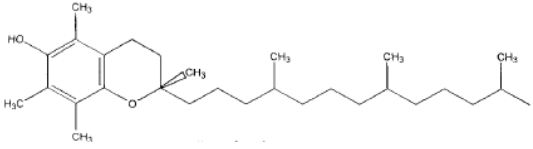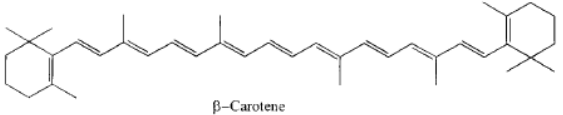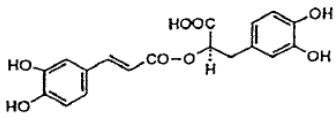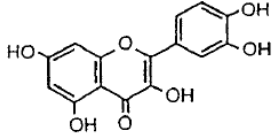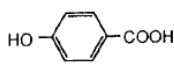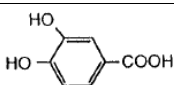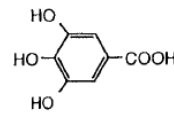Figure 2.05. Interaction of oxygen free radicals and antioxidants (Talaulikar et al., 2011)
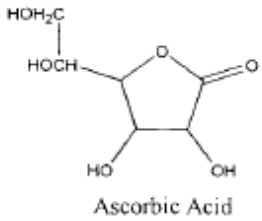
Antioxidants have a potential protective role on cardiovascular system that focused on reversing the endothelial dysfunction caused by oxidative stress (Escobales et al., 2005). Oxidative stress that cause unwanted effects on human health has become a serious issue (Krishnaiah et al., 2011).
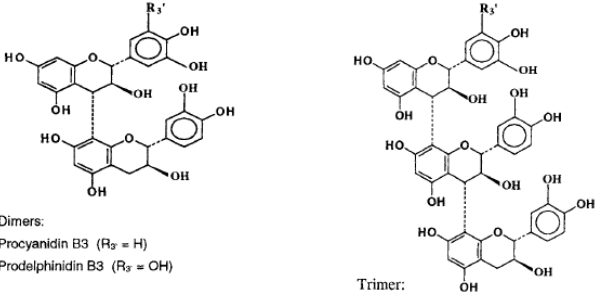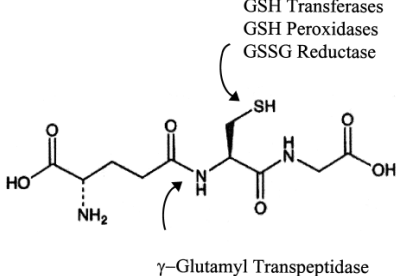
Potential antioxidants especially natural antioxidants have received great attention and have been studied in fruits, teas, vegetables, cereals and medicinal plants, as they are effective free radical scavengers and assumed to be less toxic compared to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) that were also suspected as carcinogenic agents and can cause liver damage (Zhu et al., 2011). Natural antioxidants found in foods, fruits, beverages, spices and suppliments has been investigated in numerous studies using various

methods with different conditions (Niki, 2010). Antioxidant component that known to widely distributed are various type of phenolic compounds (Okuda, 1999). Fruits and vegetables contain flavonoids and related phenolics compounds has become focused to the researcher as an addition to antioxidant vitamins and minerals (Piettea et al., 1999). Flavonoids that widely distributed in herbs and possess some polyphenolic structures might possess antioxidant activitiy such as quercetin, and their glycosides (Okuda, 1999).

The crucial role of antioxidants in the prevention, treatment of diasease as well as maintenance of human health has attract attention among researchers as the pathogenesis of various disorders and disease are involved with oxidative stress (Niki, 2010). Natural antioxidants food intake help in lowering risk factor that cause degenerative disease including cardiovascular disease and cancer (Zhu et al., 2011).

Table 2.00. Antioxidant of vitamins, polyphenol and flavonoids

Name	Chemical structure	References
α-tocopherol		Decker et al., 2000
B-carotene		Decker et al., 2000
Ascorbic acid		Decker et al., 2000
Rosmarinic acid		Okuda, 1999
Quercetin		Okuda, 1999
p-hydroxybenzoic acid		Okuda, 1999
Protocatechuic acid		Okuda, 1999
Gallic acid		Okuda, 1999

Proanthocyanidins		Pietta, 1999
Glutathione		Sies, 1999

2.4.1   Reactive oxygen species (ROS)

Free radicals and reactive oxygen species (ROS) has initiate progression of oxidative damage related disease were linked to some neurodegenerative disorders and cancers, while coronary heart disease and atherosclerosis cause by oxidation of low-density lipoprotein (Gulcin et al., 2005). The importances of Oxygen (O_2) to support life are widely known, however cell functions was modified by its metabolites such as ROS and may endanger cell survival that brings the need to continuously inactivate the ROS and to keep only a minimal amount in order to maintain normal cell function (Agarwal et al., 2003). Reactive oxygen species (ROS) known as microbicide molecules that produce by undesirable by products of areobic metabolism or by phagocytes (Yung et al., 2006). Our biological systems will produce more ROS such as superoxide anion radicals ($O2^{\cdot-}$), hydroxyl radicals (OH $^{\cdot}$) and non free radical species such as hydrogen peroxide (H_2O_2) and single oxygen ($^1O_2$) as showed in Figure 2.06, more than to produce enzymatic antioxidants and non-enzymatic antioxidants, when under stress (Krishnaiah et al., 2011). The production and metabolism of ROS were involved in the pathogenesis of cardiovascular disease including hypertension, heart failure, atherosclerosis, cardiac hypertrophy, , restenosis and dibetes mellitus (Yung et al., 2006).
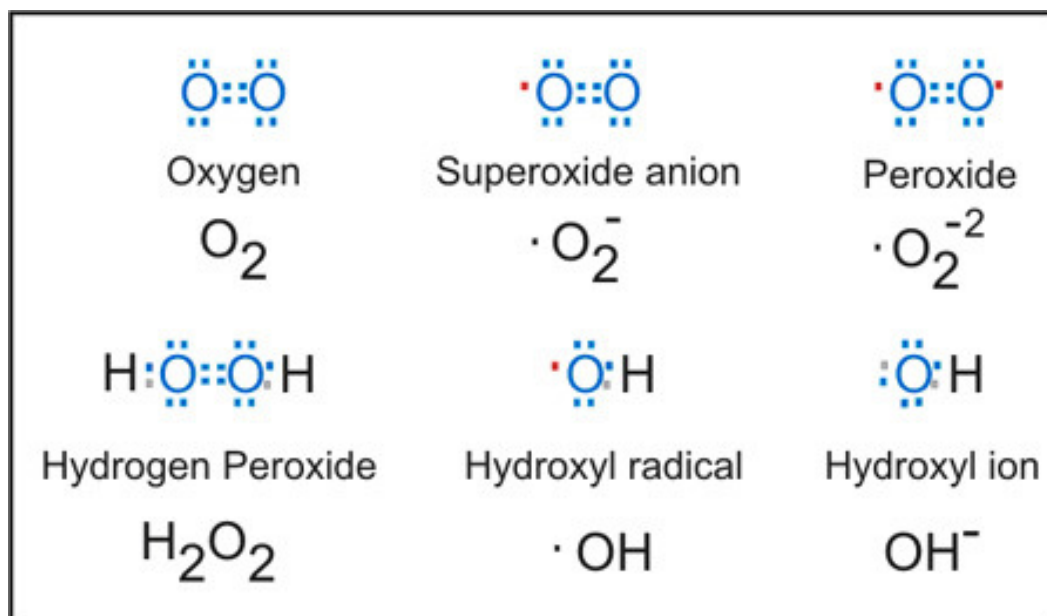
Figure 2.06. Common example of Reactive Oxygen Species (ROS) (Held, 2010).

Reactive Oxygen Species (ROS) can cause aging, cancer and other diseases such as malaria, heart disease, immunodeficiency syndrome, stroke, arteriosclerosis, diabetes and gastric ulcer by promoting oxidative damage to lipids, nulceic acid, proteins and other biomolecules such as carbohydrates (Gulcin et al., 2005). Excessive ROS production and impaired antioxidant defense mechanisms caused oxidative stress (OS) to be the cause of pathologies and known to effect the reproductive functions including a high level of toxic effects on sperm quality and function (Agarwal et al., 2003). Oxidative Stress (OS) induce DNA damage in the sperm nucleus cause acceleration of the apoptosis of germ cell leading to the decreasing in sperm counts hence deteriorated the quality of semen that associated with male infertility as well as attacks the fluidity of the sperm plasma membrane (Agarwal et al., 2003). Mutations, deletions, gene amplication and rearrangements are alterations that cause the initiation of apoptosis signalling that cause cell death or might inactivate some tumour suppressor genes and activate some of proto-oncogenes (Mate et al., 2000). ROS were reported to play an important role in apoptosis and cancer as well as promotes carcinogenesis that

will interfere with signal cascade systems including mitogen-activated protein kinases (MAPKs), activated protein-I (AP-I), phospholipase A_2, the nuclear transcription factor kappa B (NFκB), and c-Jun kinase (Mate et al., 2000). Increasing of ROS production also lead to the hypertension and vascular disease and dysfunction (Harrison et al., 2007).
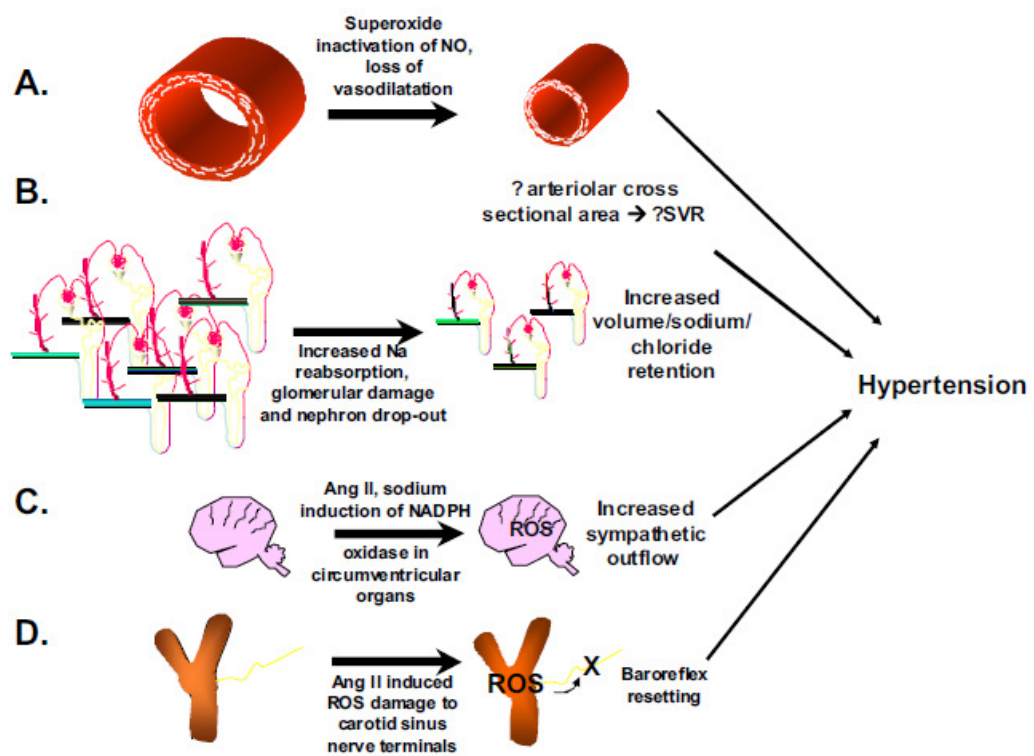

Figure 2.07. Effect of reactive oxygen species (ROS) on various organs leading to hypertension. (A) ROS inacitvate nitric oxide (NO), increase in systemic vascular resistance and cause hypertension, (B) ROS hypertension via cause nephron damage, (C) Sympathetic outflow and hypertension stimulates by ROS in the circumventricular organ, (D) alteration of the baroreflex that cause by ROS production in the vessel wall that damage afferent nerve terminals. (Harrison et al., 2007).

Figure 2.07 showed 4 factors contributed in hypertension. Superoxide inactivates the role of nitric oxide thus loss its vasodilating effects causing arteriolar cross sectional area, while glomerular damage and nephrone drop-out as well as increasing of sodium reabsorption has increased volume, sodium and chloride retention. Hypertension also occure as the sympathetic outflow increased when angiotensin II and sodium induction of NADPH oxidase in circumventricular organs while baroreflex resetting occur as angiotesin II induces ROS damage to carotid sinus nerve terminals (Harrison et al., 2007).

Primary defence system by scavenging reactive species directly includes superoxide dismutase (SOD), gluthathione peroxidase (GPX), catalase (CAT) and thioredoxin reductase is an effective strategy to prevent oxidative damage (Mate et al., 2000). As main compounds such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, vitamin A, NADPH and urate were included as secondary defence systems (Mate et al., 2000).
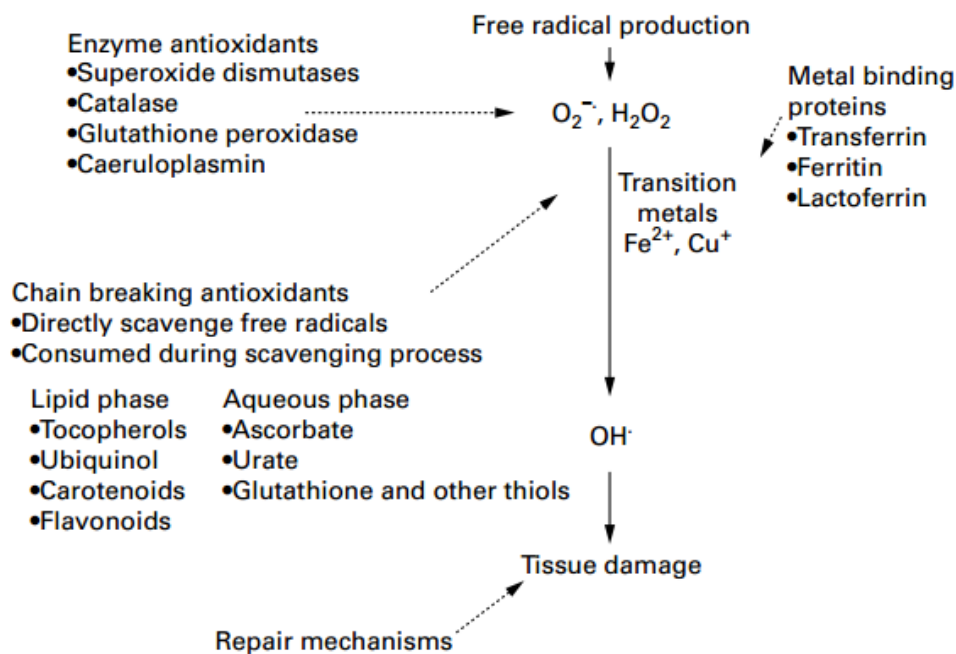
Figure 2.08. Antioxidants defense systems againts free radicals attack (Young et al., 2001).

Figure 2.08 showed the breakdown of free radicals species by antioxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase and caeruloplasmin followed by the transition metal binding proteins such as transferrin, ferritin and lactoferrin in order to prevent transition metals interacts with hydrogen peroxide and superoxide , thus prevent highly reactive hydroxyl radicals productions.

Chain breaking antioxidants reacts with free radicals and acts as powerful electron donors. It gives its electron to free radicals before target molecules are damaged. The electron transfer cause the antioxidant to oxidised, unreactive and unable to attack further molecule, thus it must be replaced or regenerated (Young et al., 2001).

2.4.2 Vitamin C

Reactive oxygen species (ROS) are widely produced in our biological systems as well as polluted environment (Drach et al., 2011). To controlled oxydation process thus to prevent membrane dysfunction, protein inactivation and DNA damage, to avoid various degenerative disease such as aging, cancer, artherosclerosis and neurodegenerative disorders, exogenous dietary antioxidants such as β-carotene, ascorbic acid (vitamin C) and α-tocopherol can be supplement to manipulate the dysfunction of antioxidant such as glutathione and glutathione-dependent enzymes, superoxidase dismutase and catalase, under certain conditions (Drach et al., 2011). In human blood, vitamin C is considered to be the most effectives aquoeus-phase antioxidant as well as a potent antioxidant for protection againts diseases and degenerative process that caused by oxidant stress (OS) (Escobales et al., 2005). Vitamin C is a water soluble antioxidant and well known as preventive and chain breaking antioxidant (Drach et al., 2011). It is highly bioavailable makes it become the most important water-soluble antioxidant in cells as well as act as efficient scavenger for ROS (Zulueta et al., 2007). It's antioxidant properties involves a hydrogen transfer rather than electron transfer (Drach et al., 2011). It improves flow-dependent dilation in forearm circulation of hypertensive patients, coronary heart disease patients, type I and type II diabetics, class III congestive heart failure patients and chronic smokers (Escobales et al., 2005).
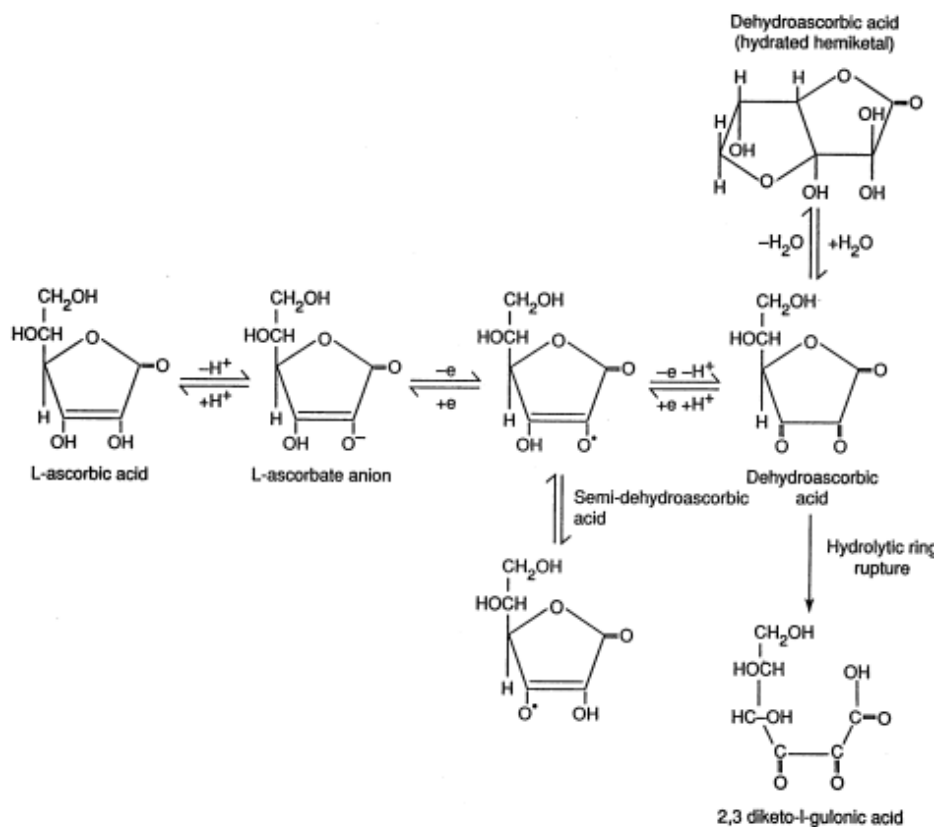
Figure 2.09. Ascorbic acid and its oxidation products (Rumsey et al., 1998).

Figure 2.09 showed chemical structure of ascorbic acid, a water-soluble antioxidants and its oxidation products L-ascorbate anion, Semi-dehydroascorbic acid, dehydroascorbic acid, hydrolytic ring rupture, 2,3 diketo-I-gulonic acid and dehydroascorbic acid. However, the formation of 2,3,-diketogulonic acid by hydrolytic ring is probably irreversible. (Rumsey et al., 1998).

**2.5   Medicinal Plant studied - *Tacca integrifolia* Ker-Gawl
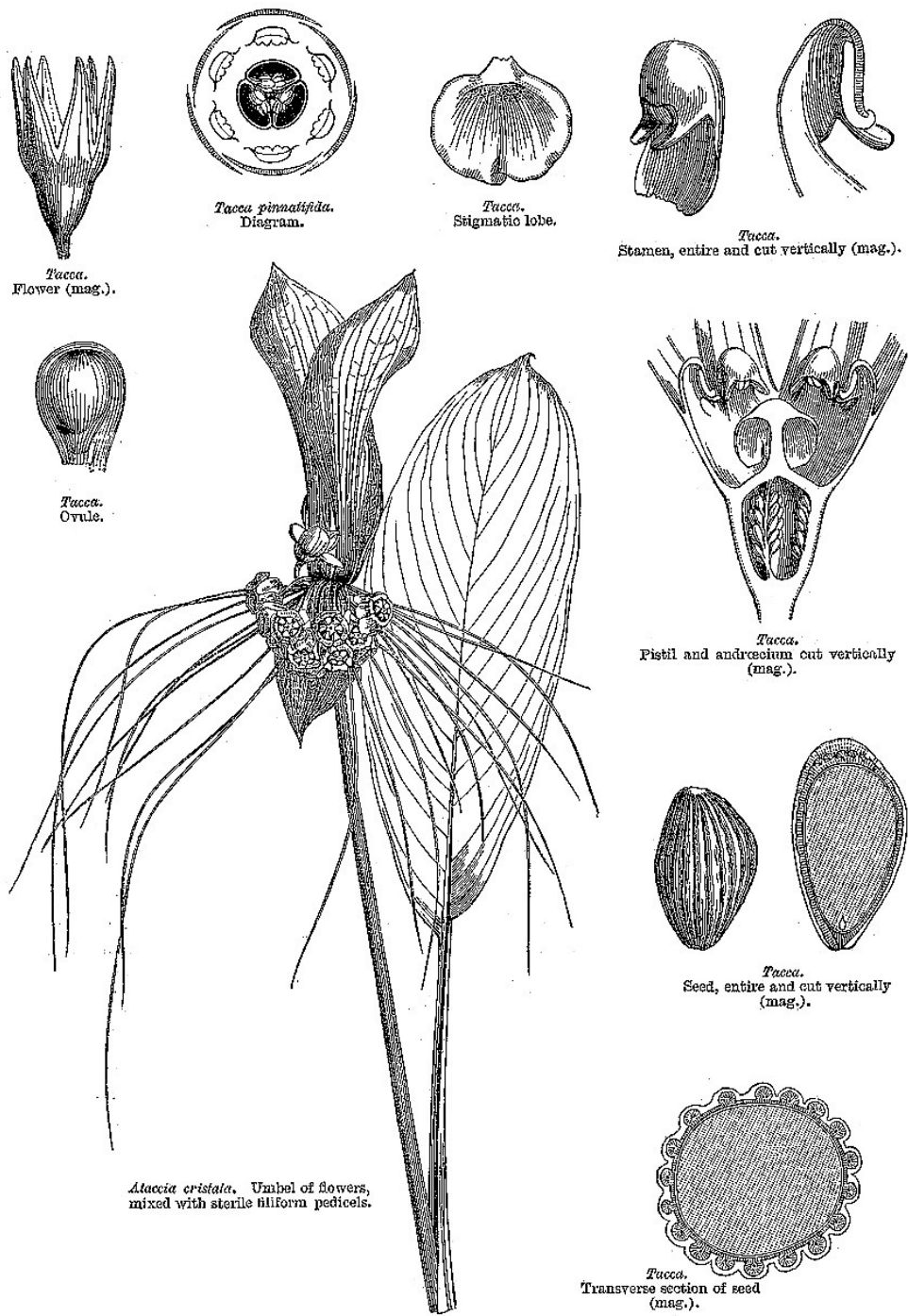   (Belimbing Tanah)**

Figure 2.10. *Tacca integrifolia*

Tacca.
Flower (mag.).

Tacca pinnatifida.
Diagram.

Tacca.
Stigmatic lobe.

Tacca.
Stamen, entire and cut vertically (mag.).

Tacca.
Ovule.

Tacca.
Pistil and andrœcium cut vertically (mag.).

Tacca.
Seed, entire and cut vertically (mag.).

Ataccia cristata. Umbel of flowers, mixed with sterile filiform pedicels.

Tacca.
Transverse section of seed (mag.).

Figure 2.11. The flower anatomy of *Tacca* sp (Watson et al., 1992)

Figure 2.12. *Tacca chantrieri* and *Tacca integrifolia* (Freeland, 2012).
1-5 *Tacca chantrieri* (1: Flowering plant, 2: Flower, 3: Open perianth showing stamens, 4: stigma and 5: Seed). 6-9 *Tacca integrifolia* Ker Gawl (6: Inner bract, 7: Flower, 8: Stigma and 9: Seed).

2.5.1   Plant Descriptions

Tacca integrifolia Ker-Gawl from family of Taccaceae is a tropical herb distributed in Southeast Asia (Razak et al., 2007). *Tacca integrifolia* is also known as *Tacca cristata* or Belimbing Tanah. In the wild, *Tacca integrifolia* live in the understory deep shade of rain forests with a diversity of soil types and a good air circulation. It has long leaves with short stemmed, and were known as rhizomatous or tuberous herbaceous plants. Four species of Asian *Tacca* have an attractive leaves and strange filiform that were look like a whisker that hang down below the flower for as much as 1 foot in length with a vertical growth habit. *Tacca integrifolia* has white bracts beautifully veined with purple, hovering over the flowers. The rhizome grows vertically with attractive leaves that emerge from the top of the rhizome and the plants can reach maximum 4' in height. As it has a beautiful flower, it has been planted in garden as ornamental plant.
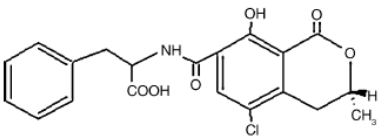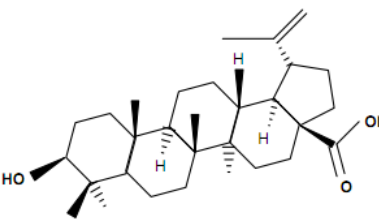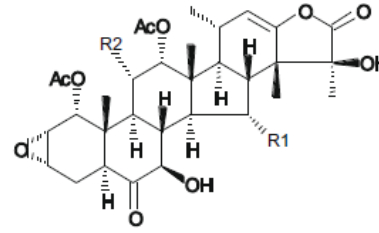
2.5.2   Medical uses

In Malaysia, the root broth of *Tacca integrifolia* has been used widely to control high blood pressure, diabetes and hemorrhoids. While woman use it as a bath to exhaust air in the body during confinement. The saps from the leaves also can be used to treat skin disease. In combination of its tuber extracts and roots that are mixed with *Goniothalamus malayanus* (Mempisang), it can be used as treatment of the kidney. *Tacca* species have been used for the treatment of gastric ulcer, enteritis, and hepatitis in China and its rhizomes are used for improving sexual function and controlling blood pressure in Thai herbal medicines (Kitjaroennirut et al., 2005).

2.5.3   Chemical constituents

Rhizome of *Tacca integrifolia* contains ochratoxin A, amino acids, n-triacontanol, castanogenin, betulinic acid, quercetin-3-α-arabinoside, and taccalin (Kitjaroennirut et al., 2005). While in other *Tacca* species such as *Tacca plantaginea*, *Tacca paxiana*, *Tacca subflabellata, Tacca leontopetaloides* and *Tacca aspera* contains anthocyanins, diarylheptanoids and diarylheptanoid glycosides such as C-27 steroidal saponins, C-28 sterol glycosides and withanolide glycosides and taccalonolides has been isolated (Li et al., 2011).

Table 2.01. Chemical compounds found in *Tacca* species

Compounds	Chemical structure	References
Ochratoxin A		Ringot et al., 2006
Betulinic acid		Subramanyama et al., 2009
Taccalonolides		Risinger et al., 2010

There are many research have been done on *Tacca sp* but there are no research has been carried out to study its antihypertensive and antioxidant properties. The main objectives of this study is to isolate and characterize the anti-hypertension properties as well as its antioxidant compounds so that continuous research can be further carried out to develop new treatment for hypertension and oxidative stress related disease that is effective and safe.

2.6   Research objectives

1.   To evaluate the phytochemical compounds in *Tacca integrifolia* extracts with the Angiotensin Converting Enzyme (ACE) inhibition.

2.   To determine the antioxidant property of *Tacca integrifolia* extracts.

3.   To investigate the effect of *Tacca integrifolia* extracts on toxicity and anti-hypertensive activity in Spontaneously Hypertensive Rats (SHR).