CHAPTER 1

INTRODUCTION

Atherosclerosis is the commonest and most important vascular disease. There are commonly confused conditions and entities between atherosclerosis and arteriosclerosis. The former implies hardening (sclerosis) or loss of elasticity of arteries due to specifically atherotoma while the latter is hardening or loss of elasticity of arteries from any cause. In medical pathological, the term atherosclerosis is an inflammatory state characterized by arterial lesions containing cholesterol and immune infiltrates with several events in early lesion development such as intimal accumulation of plasma lipoproteins, increased expression of adhesion molecules on the endothelium at sensitive sites in the vessel wall, and monocyte margination, migration across the endothelium and accumulation in the intima, where they accumulate large intracellular deposits of lipoprotein-derived cholesterol and 'fatty streak', seen in more advanced atheroma (Hansson and Libby, 2006). Many recent studies showed that the risk of developing heart disease and atherosclerosis is close related to high level of cholesterol in the blood (National Choleterol Education Program, 1990). The higher level of cholesterol in the blood, the more risk of getting a disease. Hypercholesterolemia or elevated concentration of cholesterol in the blood (Oxford Medical Dictionary, 2002) has a positive correlation to the development of various degenerative diseases namely cardiovascular disease (CVD). A high dietary intake of saturated fats and cholesterol coupled with sedentary lifestyle, which commonly practiced by urban community are the main factor for CVD. High level of cholesterol may cause increase in low-density lipoprotein (LDL) concentration in circulating blood and this condition may chronically cause the excess lipid to deposit in the vascular system and eventually promote atherosclerosis (Witztum and Steinberg, 1991). Meanwhile high density lipoprotein

(HDL) has been associated with inverse risk factor in atherosclerosis (Yusuf *et al.,* 2004).

Free radicals are factors that can lead to atherogenesis. Free radicals are atoms or molecules with one or more unpaired electrons and they are usually unstable and highly reactive. Free radicals also known as reactive oxygen species (ROS) that are produced endogenous and exogenous are superoxide anion radical O_2^-, hydrogen peroxide H_2O_2, and hydroxyl radicals OH^- (Nakazawa *et al.,* 1996). In some cases ROS such as hydrogen peroxides act synergistically with iron and copper and external factors such as ultraviolet and ionising radiations to produce free radicals that have damaging effect to the body (De Groot, 1994). In the development of atherosclerosis, lipids containing polyunsaturated fatty acids are highly sensitive to be oxidized by oxygen derived free radicals (De Groot, 1987) causing lipid peroxidation and malondialdehyde (MDA) formation thus lead to destruction of cell structures and function (Uchida, 2006). Gutteridge (1989) also showed that free radicals play an important role in the development of tissue damage and pathological events which eventually lead to cellular dysfunction and cell death.

Antioxidants in biological system capable to prevent free radicals attack on membrane lipids from oxidative damages and reduce the risk of cardiovascular disease and several aging-associated health problems such as cancer (Etsuo and Noriko, 2002). In physiological system, endogenous antioxidants that are synthesized include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GSH-Px) (Ram *et al.,* 2004) however the amount is not enough to maintain biological system as they are readily attacked by ROS and moreover under severe oxidative stress endogenous antioxidants do not sufficiently provide complete attack protection from attack of ROS (Ames *et al*., 1993). In general, antioxidant can play role by inhibit the generation of active oxidants (first line defence), scavenge and remove active oxidants

(second line defence) and detoxify, repair damage done by free radicals (third line defence). The effects of antioxidants in inhibition of atherosclerosis in hypercholesterolemic New Zealand White rabbit as animal models have been documented (Sun *et al.*, 1997).

Rhodomyrtus tomentosa or locally known as Kemunting in malay is a large evergreen shrub native originated from South East Asia (Verheij and Coronel, 1992). The flowering plant in the family of *Myrtaceae* can be found in southern India, southern China and as far as Hawaii and Florida. Derived from Greek word *Rhodon* meaning red and *myrtos* meaning myrtle and also known as Ceylon hill gooseberry, downy myrtle, feijoa, hill gooseberry, and rose myrtle. Kemunting can grow up to 12 feet tall with sharp pointed leaves about 2-3 inches long, 3 veined from the base. The blueberry-like fruits are edible, 10-15mm long. It contains sugars, vitamins and minerals. The purple pulp is juicy and sweet when fully ripe. Each *Rhodomyrtus tomentosa* flower has with five petals in clusters of two or three, 2.5-3cm diameter which are tinged white outside with purplish-pink or all pink. This type of tropical and subtropical plant often found near the coastal area and river banks especially on poor sandy soils with pH ranging from 4-10 (Ampron *et al*., 2005) and able to tolerate salty soil. Besides that the plant can also be found at the elevation up to 1000m altitude. One remarkable characteristic of this plant is fire adapted where it sprouts after fire. In Malaysia *Rhodomyrtus tomentosa* can be found in abundance mainly in the state of Kelantan, Terengganu and east coast of Johor. The plant is considered as an ornamental plant in Florida, United States and even grows invasively by competing with other native plants (Langeland and Craddock, 1998). Today there has been a wide interest in alternative medicine and natural products of plant-derived medicine studies as it carries medicinal properties such as antioxidants and vitamins. The interest has been growing as today's modern drugs are derived from non-natural products that can give unwanted

side effects. So far the cholesterol lowering effect of dietary plants has been studied from various plants and the results were promising (Hamendra and Anand, 2007; Shela *et al.,* 2003). *R. tomentosa* fruit is thought to have antioxidative effect because of rich in chemical compounds (Asadhawut and Wilawan, 2008; Dachriyanus *et al*., 2004 and Crow *et al.,* 1971). Until today, no study has been done on *R. tomentosa* on lipid profiles and lipid peroxidation in relation to cardiovascular diseases.

Research objectives

1. To identify chemical compounds in *R. tomentosa* by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and Gas Chromatography Mass Spechtrophotometry (GCMS).
2. To determine antioxidant activity of *R. tomentosa* by 1,1-diphenyl-2-picrylhydrazyl (DPPH), Metal Chelating Ability and Ferric Reducing Antioxidant Power (FRAP) assay methods, Total Flavonoid Contents (TFC) and Total Phenolic Contents.
3. To determine the toxicity effects of *R. tomentosa* extracts on *Artemia salina* and determine the maximal tolerated dose on rabbits.
4. To determine the effects of *R. tomentosa* on blood lipid profiles; total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL), blood lipid peroxidation indicated by malondialdehyde (MDA) and to observe the development of plaque formation on the aorta of rabbits fed with high cholesterol diet.

The result of this research may help to design further studies for analysis of the plant (extract) and define the components that are responsible for antioxidant actions of *R. tomentosa.* This experiment was done due to the lack of reliable studies on the use of *R. tomentosa* plant especially *R. tomentosa* fruits against lipid peroxidation and coronary diseases. Moreover this type of fruit is typically lesser known to Malaysians. *R. tomentosa* fruits may rich in chemical compounds and other biologically active components that have positive influence in health. Many epidemiological studies have strongly suggested the existence of correlation between intake of polyphenols rich foods and low mortality due to heart disease.