Cardiovascular diseases (CVD) remain the grim reaper’s primary calling card. CVDs and other related disorders are the major cause of death in the populace all over the world (Aja et al., 2015). Mortality and morbidity from CVDs, both coronary heart disease (CHD) and stroke, have declined in most high income countries and presently the largest burden has shifted to middle and low income countries (Gupta, 2014). India and China account for more cases of CVD than all developed countries put together. Unique characteristics of CVD in low and middle income countries such as India are premature onset, high mortality, increasing burden and regional variations (Gupta et al., 2012).

Conventional cardiovascular risk is attributed to lifestyle changes and altered metabolic activity. The seriousness of current scenario could be gauged by the fact that most CVD sufferers in India happen to be in their productive age which may potentially impose huge socioeconomic burden and devastating consequences over the coming years (Gupta et al., 2013). India is projected to have more deaths from cardiovascular disease than any other country in the world over the next decade. It is estimated that by 2020 cardiovascular disease will be the cause of over 40 per cent deaths in India as compared to 24 per cent in 1990 (Basu et al., 2013).

Both pharmacological and nonpharmacological strategies are likely to have a key role in tackling cardiovascular diseases. The potential for wide dissemination along with the ability to be delivered more cheaply than pharmacological strategies to both low and middle income populations is considered to be a merit for nonpharmacological strategies. Whereas, the large absolute remunerations conferred to those treated and the greater certainty in attribution of benefits are deemed to be the beneficiaries of the pharmacological strategies (Shroufi et al., 2013).

According to the Heart Disease and Stroke Statistics – 2015 Update by American Heart Association, 40.5% male and 35.5% female populations are prevalent to cardiovascular diseases (Mozaffarian et al., 2015). Venous thromboembolism (VTE) is
the third most common type of cardiovascular disease (Ansari et al., 2012). Deep vein thrombosis (DVT) mostly occurs in the legs and is associated with pulmonary embolism; collectively, these are termed VTE. The incidence of VTE in industrialized countries is 1–3 individuals per 1,000 per year. Importantly, there is a dramatic increase in the risk of VTE above the age of 50 and it reaches as high as 1 in every 100 individuals annually (Mackman, 2012). Deep vein thrombosis has serious complications such as pulmonary embolism in the acute phase and post thrombotic syndrome (PTS) in the chronic phase (Nata et al., 2013).

Hemostasis is the process that maintains the integrity of the circulatory system after vascular damage. It is a dynamic and tightly regulated process that is yet to be fully understood. Under normal circumstances, vessel wall injury rapidly initiates a series of coordinated events designed to seal the breach generated by the injury. These events lead to clot formation and require both, platelet recruitment and activation as well as the generation of thrombin and fibrin (Jalal et al., 2010).

While hemostasis represents a physiological response to prevent bleeding, the term thrombosis typically refers to the pathological formation of a thrombus (clot). A thrombus when released in the circulatory system is often termed as embolization, thereby reduces or ceases the blood flow to a tissue resulting in severe consequences (Ali et al., 2013). Thrombosis is the fundamental pathophysiological process that underlies the acute coronary disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks (Dewan and Das, 2013).

Despite the success of interventional trials, the real world of atherothrombosis is complicated, with a high rate of morbidity and mortality. Several issues related to the antiplatelet treatment may account for cardiovascular relapses (Violi et al., 2010). However, recognition that lysis of preformed fibrin could be accomplished in vivo by a process involving the conversion of inactive plasminogen to active plasmin enzyme led to an alternative enzyme based approach (Mushtaq and Jamil, 2012).

The era in which vitamin K antagonists were the only option for long-term anticoagulation has ended. Patients now have multiple treatment options for prophylaxis for nonvalvular atrial fibrillation and prevention and treatment for venous thromboembolism. Novel oral anticoagulants, consisting of direct thrombin inhibitors and factor Xa inhibitors are a diverse group of agents that have reduced medication and food interactions compared to warfarin and they eliminate the need for frequent monitoring (Raja and Geyer, 2013). Historically, the most commonly used antithrombin agent for
Percutaneous coronary intervention (PCI) is unfractionated heparin (Rao and Ohman, 2010). They are used for the prevention and initial treatment of thromboembolism and during revascularization (Mongale et al., 2012).

Thrombolytic therapy, also known as clot busting, is used if the drug can be administered within 12 hours of the onset of symptoms. The patient is eligible based on exclusion criteria and if primary PCI is not immediately available. The effectiveness of thrombolytic therapy is the highest in the first two hours after a heart attack. Irreversible injury to the heart muscle occurs within two to four hours of the heart attack due to a lack of blood flow and oxygen and there is a limited window of time available for reperfusion to work (www.wellness.com).

Thrombolytic drugs like tissue plasminogen activator (tPA), urokinase, streptokinase etc. play a crucial role in the management of patients with cerebral venous sinus thrombosis (Chowdhury et al., 2011). Only three agents have been approved for use in the United States: streptokinase, urokinase and tissue-type plasminogen activator. Urokinase is not currently available for use in the United States. The latter agent has been most widely used on the basis of proven benefit with a relatively short (2-hour) infusion. Newer, unapproved agents include tenecteplase and reteplase. Risk stratification in acute pulmonary embolism is important in determining which patients are the most appropriate candidates for thrombolysis, with careful consideration of contraindications (Tapson, 2013).

All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity, severe anaphylactic reactions and bleeding tendency. Attempts are underway to develop improved recombinant variants of these drugs to overcome the inadequacies. Oral administration of nattokinase was tried as a preventive measure against thrombosis which was reported to enhance fibrinolytic activity in plasma (Elumalai et al., 2012).

Most of the developing countries and health insurance companies do not cover the high cost of thrombolytic therapy for stroke patients (Pandian et al., 2007). Prehospital delay, financial constraints and lack of infrastructure are main barriers of thrombolytic therapy in developing countries. Instead, developing countries should focus on primary and secondary stroke prevention strategies until a proper infrastructure for therapy and a cheaper source for thrombolytic agent is available. However, governments
and health systems of developing countries should exert efforts for promotion of their infrastructure of stroke care (Ghandehari, 2011).

In the reperfusion therapy of acute myocardial infarction, combination therapy is most often used because ischemic rat models have proved an increase in oxidative stress during reperfusion (Domínguez et al., 2010). Hence, any thrombolytic agent that can pledge both antioxidant potential and thrombolytic property would be the choice of interest. Oral anticoagulants are simpler to administer, can be given in fixed doses and offer the most promise. It increases the uptake of anticoagulant prophylaxis in patients with stroke thereby decreasing the death and disability (Eikelboom and Weitz, 2010).

Drugs based on herbs have become a common form of therapy because they are often perceived as being natural and therefore harmless. Today, they are one of the hottest trends and most sought after in the field of nutrition or herbal therapeutics (Singh et al., 2012). In recent times, there has been a shift in universal trend from synthetic to herbal medicine due to side effects of synthetic products which we can say “Return to Nature” (Sharma et al., 2008).

India is sitting on gold mine of well recorded and traditionally well practiced knowledge of traditional herbal medicine (Kamboj, 2010). Nature has bestowed our country with an enormous wealth of medicinal plants and India has often been referred to as the “Medicinal Garden of the World” (Verma et al., 2012). But unfortunately, India has not done well in the International trade of herbal industry due to lack of scientific input in herbal drugs. So it is necessary to integrate modern knowledge with traditional system of medicine (Arya et al., 2013).

Traditional medicinal plants use in India is about 4000 years old. Herbs had been used by all cultures throughout history. It was an integral part of the development. About 80% of the people in developing countries use traditional medicines for their health care. In less developed/developing countries 80% of the people still rely only on traditional medicine obtained from local plants and 85% of traditional medicine involves the use of plant extracts (Sivasankari et al., 2013).

Along with the nutritional value, plants contribute in protection from free radical deterioration by hindrance of lipid peroxidation via numerous mechanisms including scavenging free radicals, inducing antioxidant enzymes, modulating protein kinase and lipid kinase signalling pathway. Oxidative damages done by free radicals cause
pathogenesis of many of the deadly diseases like cancer, Alzheimer’s and diabetes (Sen and Chakraborty, 2011).

Advances of phytochemistry and identification of plant compounds which are effective in curing certain diseases have renewed the herbal medicines (Arifuzzaman et al., 2011). Phytochemicals of varied chemical structures from fruits and vegetables have already been studied extensively for their potential anticancer or chemopreventive efficacy. Being the rich sources of vitamins, minerals and fiber without posing “any side effects” made fruits and vegetables the best choice to lower the cancer risk and also in maintaining good general health (Tayarani-Najaran and Emami, 2011).

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene need to be replaced with natural antioxidants due to their potential health risks and toxicity. Sources of natural antioxidants are mainly plant phenolics found in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Anjum et al., 2013).

The important role of plant derived compounds is undeniable. About 79% of the medicinal plants show some cytotoxicity, while 75% of the nonmedicinal plants exhibit bioactivity (Booth et al., 2012). Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose (Uddin et al., 2014).

Herbal preparations on the other hand, if taken in appropriate doses, can lead to an alternative and better option for curing various ailments. Although, toxicities of many plant extract are a major concern, the development of methods for lethality assay has been successfully used to biomonitor the cytotoxicity of plant materials (Al-Mamun et al., 2012). Thus, in vivo lethality of an extract against a simple zoological organism, brine shrimp nauplii (Artemia salina) or any cell line can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products (Chakma et al., 2013).

Phenolic compounds widely distributed in plants, attract significant scientific interest due to their biofunctional health promoting properties. Fruits are potential sources of natural phenolic antioxidants and are used as food additives for the prevention of lipid oxidation. Many fruits have been characterized for their phenolic profile and antioxidant activity (Maqsood et al., 2013). Polyphenolic compounds consist of different phenolic rings, out of which one of the major subgroups of these secondary
metabolites are flavonoids. They are widespread in nature and are consumed as part of the human diet in significant amounts (González et al., 2011).

The decreasing efficacy of some synthetic drugs and the increasing contraindications of their usage make the practice of natural drugs topical again. Thus, the study of phytotherapy for chronic diseases treatment might yield an excellent return in potential sources of medicinal plants which play vital roles in disease prevention and their promotion and use, fit into all existing prevention strategies (Eddouks et al., 2014).

A number of epidemiological trends and clinical studies support the notion of a diet rich in fruits and vegetables being correlated with reduced cardiovascular complications and mortality. Polyphenol rich diets have been associated with reducing CVD risk thereby promoting optimal ageing. Plant based low carbohydrate diet was seen associated with lowering the cardiovascular mortality (Khurana et al., 2013).

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Kiranmai et al., 2011).

Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet, anticoagulant, antithrombotic and thrombolytic activity. Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could reduce the risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations also have been reported (Hossain et al., 2012). Further studies are required for the exploration of isolated molecule that can be effective, safer, cheaper and nontoxic enough for ameliorating the thrombosis conditions (Sherwani et al., 2013).

So far, there are not much systematic reports on the “Evaluation of the thrombolytic potential of Punica granatum by in vitro, in vivo and in silico approaches” in a systematic and scientific manner.
The study was performed in five different phases with the following objectives:

- To screen diverse plant based sources for thrombolytic activity
- To evaluate the antioxidant potential of the selected plant source
- To isolate the principle compound responsible for thrombolysis and to determine the efficiency in vitro
- To assess the in vivo clot lysing efficacy of the selected extracts in rats
- To analyze the interaction of active compounds present in the sample with thrombolytic targets in silico

**HYPOTHESIS:**

The study focussed on the efficiency of aril and rind of the fruit *Punica granatum* to lyse the blood clots that lead to cardiovascular diseases.

Hence the null hypothesis proposed for the study was:

- The aril and rind of *P. granatum* do not exhibit thrombolytic efficiency for lysing the blood clot.

The alternate hypothesis proposed was:

- The aril and rind of *P. granatum* do have an influential role in lysing the clot through thrombolytic pathway.

Literatures available pertaining to the present study was collected and examined and is present as concise review in the next chapter.