

## **2.0 REVIEW OF LITERATURE**

The review of literature pertaining to the study “**Evaluation of Thrombolytic and Antioxidant Potential of *Moringa Pterygosperma Gaertn.***” are discussed under the following headings:

### **2.1 Medicinal plants**

### **2.2 Green leafy vegetables**

### **2.3 Phytochemical components**

### **2.4 Thrombosis and Cardiovascular diseases**

### **2.5 Thrombolysis and Thrombolytics**

### **2.6 Medicinal plants for thrombolysis**

### **2.7 Antioxidants**

### **2.8 Structural studies**

### **2.9 Molecular modeling**

### **3.0 Medicinal property of the selected plant**

#### **2.1 Medicinal plants:**

Plants are a great source of medicines, especially in traditional medicine, which are useful in the treatment of various diseases. Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals (Verma *et al.*, 2012). There is a great demand for herbal medicine in the developed as well as developing countries, because of their wide biological activities, higher margin of safety than the synthetic drugs and lesser costs. India is sitting on gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. The country is perhaps a great source of medicinal species and is rightly called the botanical garden of world owing to its rich biodiversity. It should be aimed to bolster interest in this field by carrying out scientific research into promising herbs that are based on nutraceuticals. Of necessity, such research eventually focuses on finding of active ingredients and this has led to the development of isolates from plants as "nutraceuticals" (Parveen and Shrivastava, 2012).

The properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians in Ayurveda, which is a basic foundation of ancient medical science in India. Several investigations have shown that many of these plants have antioxidant activities that could be therapeutically beneficial and it has been mentioned that the antioxidant potential of plants might be due to their phenolic components (Beidokhti and Prakash, 2013).

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds . Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances. (Yadav and Agarwala, 2011).

## **2.2 Green leafy vegetables:**

The importance of fruits and vegetables in human diet is well established. Plant foods are low in fats and energy; they are good sources of vitamins, minerals and polyphenols and provide a significant amount of fibre. These properties make them highly recommended for daily diet. When consumed in a proper way, fruits and vegetables offer many health benefits, including reduction of body weight, reduction of the risks of type 2 diabetes, cardiovascular disease and certain types of cancer. The majority of noticed health benefits of fruits and vegetables are attributed to their bioactive ingredients and their specific combinations (Dodevska *et al.*, 2015). Some plant chemicals are capable of treating inflammatory disorders and eliminate carcinogens, while many other leafy vegetables regulate the rate at which cells reproduce, get rid of old cells and maintain DNA. Leafy greens also contain a lot of water, which contributes to beautiful skin and hair one should consume a diet rich in B vitamins like salmon, sardines, legumes and whole grains, along with fortified cereals and breads, seeds and dark green leafy vegetables (Settaluri *et al.*, 2015).

Green leafy vegetable consumption has been associated with decreased risk of persistent metabolic diseases. There are 45,000 species of wild plant out of which 9,500 species are ethnobotanically important species. Of these, 7,500 species are in medicinal use for indigenous health practices. About 3,900 plant species are used by tribals as food out of which 521 species are leafy vegetables (Kamble and Jadhav, 2013).

The biochemical components present in green leafy vegetables are of great pharmacological or medicinal importance. The phytonutrients present in green leafy vegetable give many common health benefits such as protection from eye disorders, oxidative stress, iron deficiency etc., Consumption of green leafy food is good for human health as it improves nutritional status and reducing risks of specific diseases like diabetes, cancer and hepatotoxicity. Fruits and vegetables are important sources of phytochemicals and it is studied that some antinutritional content of these vegetables have exhibited potential for reducing the risk of certain diseases in human beings. These diseases include high blood pressure, heart attack, stroke and other cardiovascular diseases (Mane *et al.*, 2015).

### **2.3 Phytocomponents:**

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Mir *et al.*, 2013). The value of natural products in the treatment of ailments is well-known. Amongst the various natural sources, plants are an important source of bioactive constituents, including anticancer, antifungal and antimicrobial drugs. More than 1000 plant species are known for their anticancer potential. The use of plant compounds as prototypes of new drugs has a historical and economic importance (Satyavathi *et al.*, 2015).

Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region. Following such leads, plant parts are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization.

Then it can be used as the basis for a new pharmaceutical product (Ugochukwu *et al.*, 2013).

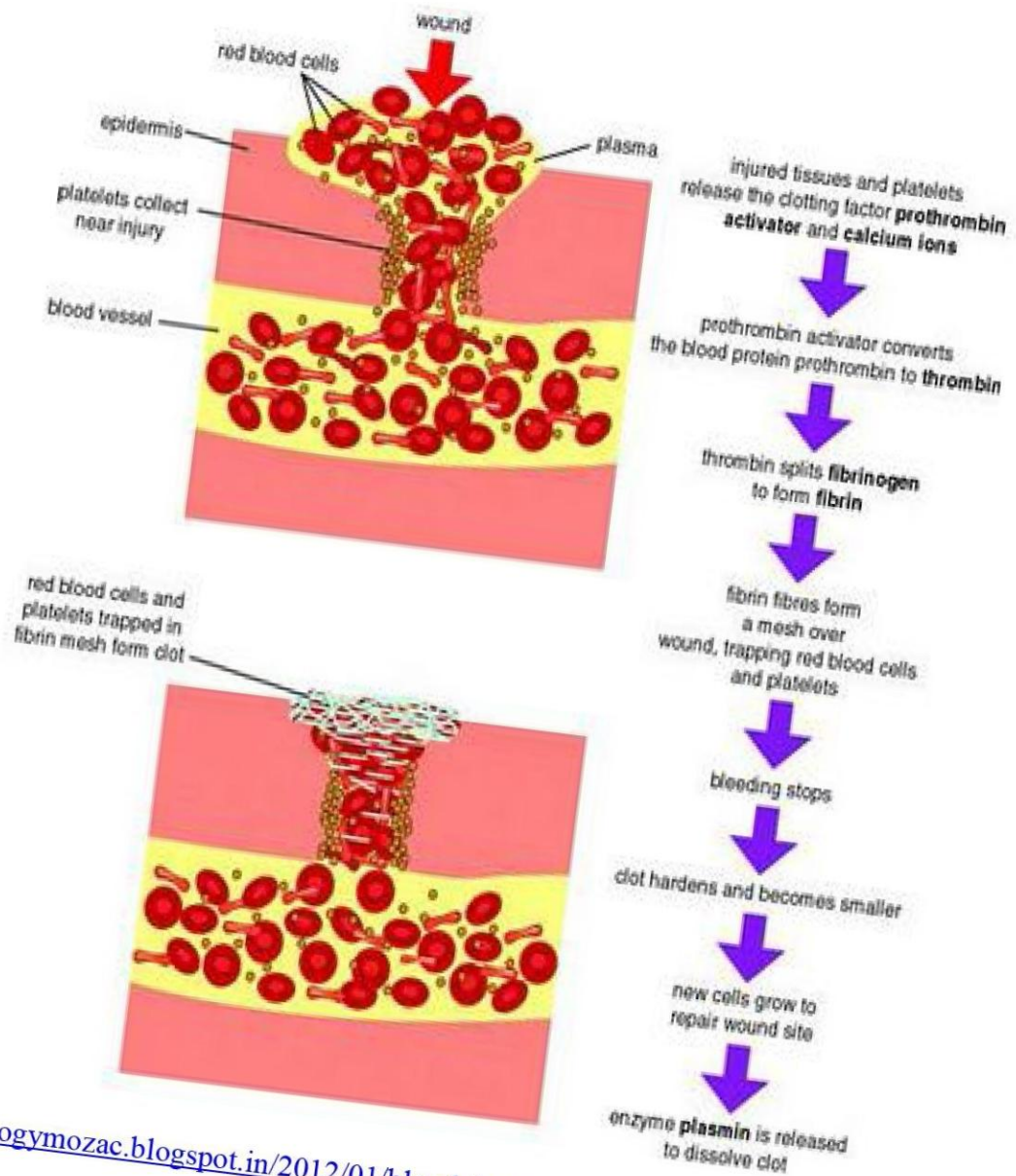
Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years and continue to play a major role in the primary health care of about 80% of the world's inhabitants. The compounds important in maintaining human health are referred to as "bioactive compounds and these include flavonoids, glucosinolates, carotenoids, organosulfides, sterols and peptides. Plant-derived antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits (Sowmia *et al.*, 2014).

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom and about 3000 varieties of flavonoids are known, which are classified as flavones flavanones catechins and anthocyanins. Flavonoids have different pharmacological and biological activities (Agarwal *et al.*, 2011).

Since ancient times plants have served as a natural source of treatment and therapy. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds. In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Sakib *et al.*, 2015).

#### **2.4 Thrombosis and Cardiovascular diseases:**

Thrombosis is the process of formation of solid mass or thrombus in circulation from the constituents of flowing blood. A blood clot is the mass of coagulated blood formed *in vitro*. In other words, haemostatic plug at the cut end of a blood vessel may be considered the simplest form of thrombosis. Haemostatic plugs are useful as they stop the escape of blood and plasma, whereas thrombi developing in the unruptured cardiovascular system may be life threatening by causing ischaemic injury and Thromboembolism (Dar *et al.*, 2013).



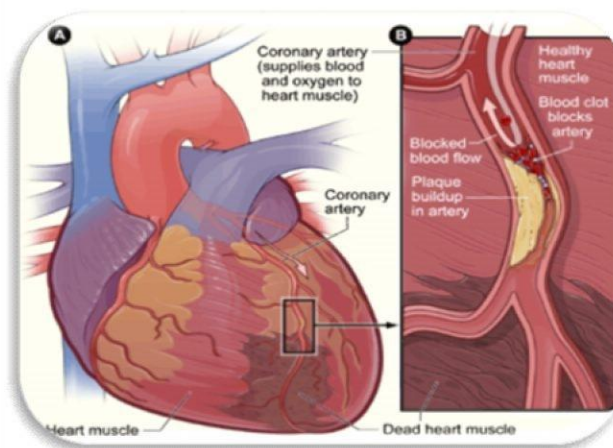
( <http://biologymozac.blogspot.in/2012/01/bloodclotting-mechanism-flow-chart.html>)

Diseases arising from clots in blood vessels include pulmonary emboli, deep vein thrombosis, CVD and myocardial infarction which are the major causes of mortality and morbidity. These disorders collectively are the most common cause of death and disability in the developed world. Hemostasis is the process that retains the blood within the vascular system during periods of injury. The coagulation mechanism may be thought of as a complex series of cascading reactions involving development of enzymes from their precursor (zymogens, procoagulants proenzymes). Most of the substances which are necessary for coagulation are present in an inert form and must be converted to an activated state. As one enzyme is formed it then becomes available to convert the next zymogen to its activated enzyme (serine protease). This process continues until a fibrin meshwork clot

is formed. In addition to the zymogens, protein cofactors and surface membrane phospholipids and calcium ions play an active role in the final development of the fibrin clot. (Jain *et al.*, 2014)

Cardiovascular disease and other related disorders are the major cause of mortality or death in the populace all over the world-both in developed and developing countries. In the ethnology of cardiovascular disease, high level of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) in the serum have been implicated and seen as primary risk factor. A number of epidemiological investigations have shown a clear association between dietary saturated fat, atherosclerosis and coronary heart disease (CHD) (Aja *et al.*, 2015). Atherosclerotic cardiovascular disease (AVD) is the leading cause of death and morbidity in the western world. Since first proposed in 1999 by Ross, inflammation has been gradually accepted to be one of the key components in development of atherosclerosis and in its acute clinical manifestations such as heart attacks and strokes (Wang *et al.*, 2013).

Cardiovascular disease (CVD) is considered as the leading cause of death and 80% of CVD related deaths have been reported from low and middle income countries like India. It is expected that by 2020, CVD would prevail as the leading cause of death and disability over infectious diseases globally. Cardiovascular diseases encompass atherosclerotic vascular diseases like coronary heart disease (CHD), cerebrovascular disease (CBVD) and peripheral arterial diseases. The most commonly studied emerging cardiovascular risk factors included coronary artery calcium score, lipoprotein (a), apolipoproteins, homocysteine, thrombosis markers like fibrinogen and plasminogen activator inhibitor 1, carotid intima-media thickness, genotypic variations, non alcoholic fatty liver disease, C-reactive protein, platelets and birth weight levels (Gupta *et al.*, 2014).



Platelet aggregation plays a central role in thrombosis (clot formation). The presence of a thrombus in an artery providing blood to the heart is the most common cause of acute coronary syndromes such as myocardial infarction and angina. Inhibitors of aggregation can provide protection against these symptoms that affect millions of people worldwide. Acetylsalicylic acid (aspirin) is one such inhibitor. The chances of a second heart attack can be reduced by as much as 40% by taking aspirin daily. Increasing the level of natural platelet inhibitors in the diet may also reduce the risk of developing cardiovascular disorders mediated by platelet aggregation (Briggs *et al.*, 2000).

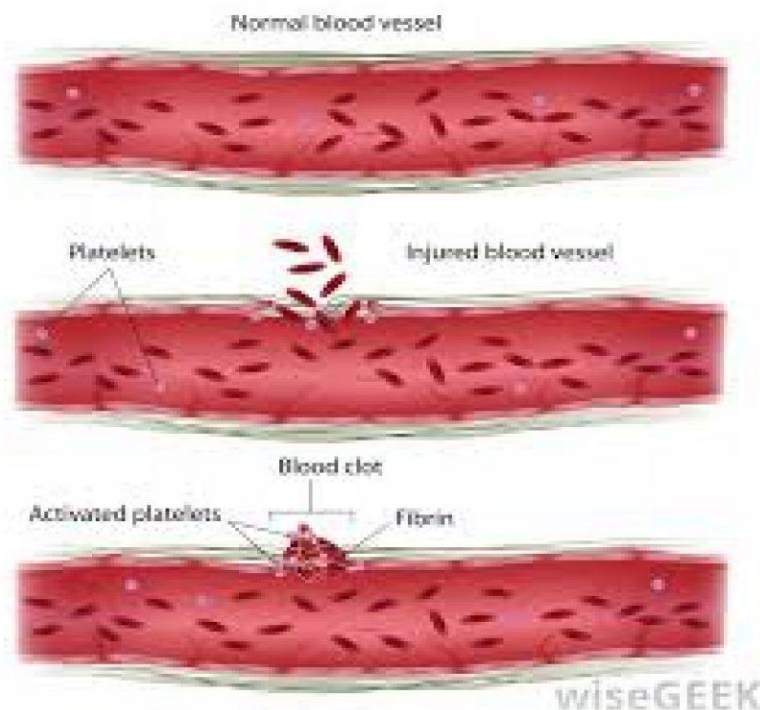
## **2.5 Thrombolysis and Thrombolytics:**

A blood clot (thrombus) developed in the circulatory system due to the failure of hemostasis causes vascular blockage and leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death (Sayeed *et al.*, 2014). The coagulation itself is termed a thrombus. Medicinal plants assume a predominant part in the treatment of mixtures of human ailments from the dusk of the human development (Tarek, *et al.*, 2015). Mainly there are two types of thrombosis based according to the site of clot formation and these are venous thrombosis and arterial thrombosis (Chowdhury *et al.*, 2015). Thrombolytic therapy consists of the administration of a pharmacological agent to cause thrombolysis of an abnormal blood clot. Thrombolytic therapy is also used to dissolve blood clots that form in catheters or tubes put into people's bodies for medical treatments, such as dialysis or chemotherapy. When a blood clot forms in a blood vessel, it may cut off or severely reduce blood flow to parts of the body that are served by that blood vessel. This event can cause serious damage to those parts of the body. If the clot forms in an artery that supplies blood to the heart, for example, it can cause a heart attack. A clot that cuts off blood to the brain can cause a stroke. (Siddika *et al.*, 2015).

Treatment of thrombosis is directed against further thrombus formation and commonly performed with unfractionated heparin or low-molecular weight heparin. Although recombinant hirudin, a specific thrombin inhibitor that dissolves thrombi, has been investigated experimentally, thrombolytic agents are not commonly employed in equine medicine. Tissue-type plasminogen activator (t-PA) is an endogenous enzyme that induces lysis of intravascular thrombi by the activation of plasminogen to plasmin, which degrades the fibrin components of blood clots (Bäumer *et al.*, 2013).

Thrombolytic agents are used to treat a wide variety of venous and arterial thromboembolic disorders, especially against acute myocardial infarction. These are normally plasminogen activators that make use of the vascular system's own intrinsic thromboresistance defense mechanism by accelerating and amplifying the conversion of an inactive precursor, plasminogen to the active enzyme plasmin, a natural fibrinolytic agent, which in turn hydrolyzes several key bonds in the fibrin (clot) matrix causing dissolution. Thrombolytic agents can be categorized in several ways. Classification schemes can be devised on the basis of the source of the agent, the propensity for enhanced enzymatic activity on a fibrin or cell surface or the mechanism of action (enzymatic vs. non-enzymatic) or different generation wise (Rao *et al.*, 2010).

Thrombosis, or blood clot formation and its sequelae remain a leading cause of morbidity and mortality and recurrent thrombosis is common despite current optimal therapy. Protein disulfide isomerase (PDI) is an oxidoreductase that has recently been shown to participate in thrombus formation (Jasuja *et al.*, 2014).



## 2.6 Medicinal plants for thrombolysis:

Medicinal plants are one of the important contributors to the most of the medicinal preparations as raw plant materials, refined crude extracts and mixtures etc. Several thousands of plants have been identified containing medicinal values and are used to treat

different ailments in various cultures worldwide. Even in this modern world, majority of the people are still relying on the traditional medicine for their primary health care. (Laboni *et al.*, 2015).

Natural products have earned an admirable place in drug discovery. Bangladesh is a good repository of natural products. Numerous plants are available to facilitate the traditional treatments. Proper scientific evaluations are essential to explore the plant derived drugs (Dey *et al.*, 2014). The evaluation parameters for plant based drugs as per official guidelines are, physical, chemical and biological evaluation (Kolpe *et al.*, 2014).

Some plants or plant parts showing thrombolytic activity have also been reported . *Trema orientalis* (Chikan), *Bacopa monnieri* (Brahmi), *Capsicum frutescens* (Lankamarich or Marich), *Brassica oleracea* (Cauliflower), *Urena sinuate* (Kunjia, Kungooya) are native to Bangladesh. They are used as traditional medicines for cardiac diseases and blood purification (Emran *et al.*, 2015).

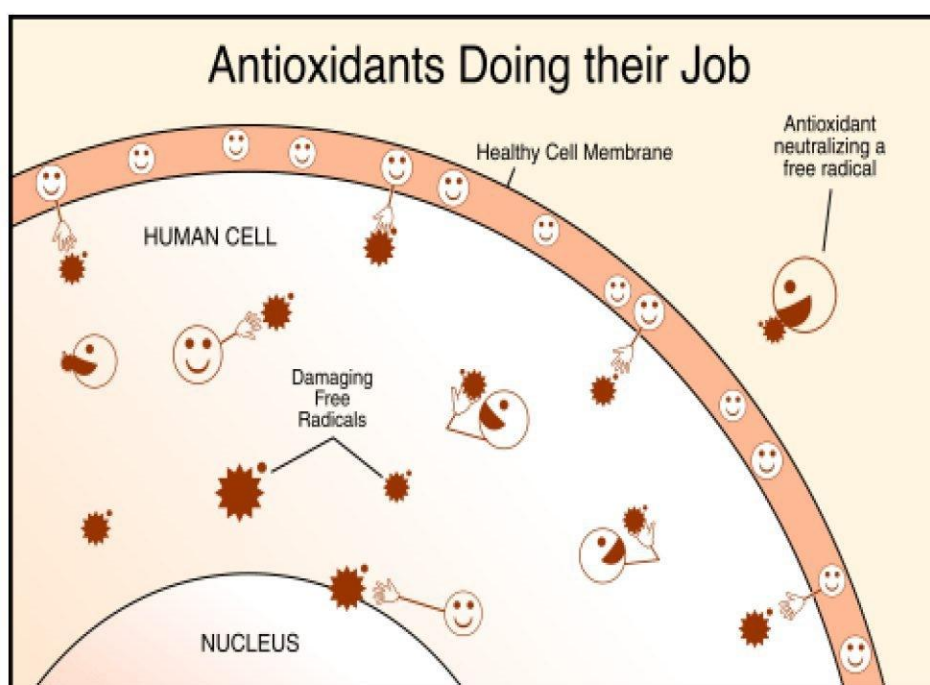
### **Antioxidants:**

Living organisms are equipped with a defense system to neutralize free radicals and other reactive oxygen species (ROS). This defense system includes enzymic antioxidants such as catalases, superoxide dismutase, glutathione peroxidase, glutathione reductase; and non- enzymic antioxidant namely glutathione, vitamins E and C, etc (Rezaeian *et al.*, 2015). Antioxidant plays an important role in inhibiting and scavenging free radicals, thus providing protection to human against infection and degenerative diseases. Over the past few years, a number of medicinal plants have been investigated for their quenching activity of specific ROS. Interestingly, these medicinal plants contain large amounts of antioxidants (Karnan and Subramani, 2015). They can also provide food protection against oxidative degradation, hence antioxidants increase food quality and acceptability (Haghju and Almasi, 2015).

Antioxidants are classified as natural and synthetic which are further divided in primary and secondary antioxidants. Plants are the source of natural antioxidants like ascorbic acid, carotenoid, tocopherol (Anjali and gaytari, 2015). Most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA, etc.) have reported to cause side effects or negative health effects. There is need to replace the synthetic antioxidants with natural antioxidants due to their potential health risks and toxicity (Pawar *et al.*, 2015). Studies on the antioxidant contents of fruits and vegetables

are increasing because natural antioxidant consumption has been found to be related with decreased risk for cancer and heart diseases (Mashkor, 2015).

Free radicals are molecules or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and are capable of independent existence and are involved in the normal physiology of living organisms. Under certain conditions, the excess of free radicals and Reactive Oxygen Species (ROS) like peroxy radical ( $\text{ROO}\cdot$ ) have been proposed to induce cellular damage and to be involved in several human diseases (Ibraheim *et al.*, 2015). Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as Parkinson's disease, Alzheimer's disease, cancers, aging, coronary, heart ailments, cardiovascular diseases, atherosclerosis, cataracts and chronic inflammatory diseases and other degenerative diseases. Medicinal plants contain many antioxidants such as vitamins, carotenoids, flavonoids, polyphenols, saponins, enzymes and minerals. Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, an hepatoprotective properties (Irimpan *et al.*, 2012).



### Structural studies:

It is necessary to develop a method for rapid, precise and accurate identification and estimation of active constituents or a marker compound as the qualitative and quantitative target to assess authentic and inherent quality. Through various analytical techniques like

TLC, HPLC and HPTLC we the presence of these compounds in plants and also quantify the. HPTLC offers many advantages over other chromatographic techniques such as unsuppressed flexibility, choice of detection, user friendly, rapid and cost effective. This HPTLC is widely used at industrial level for routine analysis of herbal medicines (Sutar and Pal, 2015).

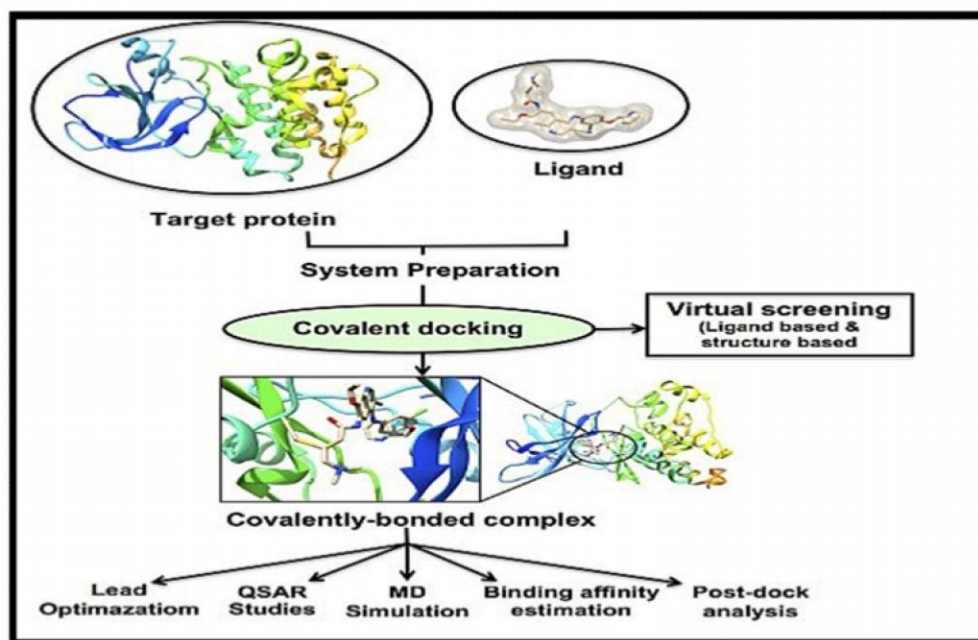
In recent years, although technology and medicine have developed extensively due to decrease in natural richness and other drawbacks. A variety of techniques can be used to determine and estimate the presence of such phytochemical compounds. Various chromatography methods like High Pressure Liquid Chromatography (HPLC) and Thin layer Chromatography (TLC) are commonly used. Chromatography is an analytical technique dealing with the separation of closely related compounds from a mixture. Various chromatography methods like Thin layer chromatography (TLC) and High Pressure Liquid Chromatography (HPLC) are commonly used. Thin layer Chromatography in one and two-dimensional (1/2D) modes is a powerful technique, which has been used successfully in the separation and determination of a large number of saponins in plant extracts (Sharma and paliwal *et al.*, 2013).

## **2.9 Molecular modelling:**

Molecular modeling is the new technique which considers molecule as model and predict its interactions with target molecules. In case of molecular “docking” ligand molecule is docked inside cavity of protein so as to mimic natural course of interaction of ligand and receptor via lowest energy pathway. Docking is a method which predicts the preferred orientation of one molecule into another when bound to each other to form stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring function (Londhe, *et al.*, 2015). Ligand is a small molecule, which interacts with protein’s binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes (Vijesh *et al.*, 2011).

Molecular docking is the most commonly used technique in the modern drug discovery process where computational approaches involving docking algorithms are used to dock small molecules into macromolecular target structures. Over the recent years several evaluation studies have been reported by independent scientists comparing the

performance of the docking programs by using default ‘black box’ protocols supplied by the software companies. Such studies have to be considered carefully as the docking programs can be tweaked towards optimum performance by selecting the parameters suitable for the target of interest. (Udatha *et al.*, 2012).



### 3.0 Plant selected for the study:

*Moringa pterygosperma* Gaertn. (Moringaceae), native to the western and sub Himalayan region, India, Pakistan, Africa and Arabia is now distributed in the Philippines, Cambodia, Central North and Caribbean Island . The *Moringa* tree is cultivated and used as a vegetable (leaves, green ponds, flower, roasted seed), for spice (roots), for cooking and cosmetic oil (seeds) and all plant organs have medicinal properties. It has an impressive range of medicinal uses with high nutritional value. Different parts of these plants contain a profile of important minerals and are a good source of protein, vitamins, beta carotenes, amino acid and various phenolic compounds. Its leaves have the calcium equivalent of four times that of milk, the vitamin C content is seven times that of orange while its potassium is three times that of bananas, three times the iron of spinach, four time, the amount of vitamin A carrotsand two time the protein in milk. Besides, *Moringa* is also suggested as a viable supplement of dietary minerals (Bargah, 2015).

*Moringa pteygosperma* Gaertn. (Moringaceae) is a highly valued plant, A wide range of chemical constituents including quercetin, kaempferol, alkaloid, vanillin, 4-hydroxymellin and octacosanoic acid and terpenoids are present in this species. Various

parts of this plant such as the leaves, roots, seeds, stem bark, fruit and flowers can be used as cardiac and circulatory stimulants, antitumor, antipyretic, analgesic, antiulcer, antispasmodic, anti-inflammatory, diuretic, antioxidant, antidiabetic, antibacterial, antifungal agents and are being employed for the treatment of different ailments in the indigenous system of medicine (Bargah and Das, 2014).



#### Scientific classification

Kingdom	:	Plantae
Order	:	Brassicales
Family	:	Moringaceae
Genus	:	Moringa
Species	:	<i>M. oleifera</i>
Binomial name	:	<i>Moringa Pterygosperma</i> Gaertn.

A number of medicinal properties attributed to different parts of *Moringa* have been recognized by both Ayurvedic and Unani systems of medicines. The plant finds its wide applicability in the treatment of cardiovascular diseases. The roots, leaves, gum, flowers and infusion of seeds have nitrile, mustard oil glycosides and thiocarbamate glycosides as their chemical constituents which are suggested to be responsible for the diuretic, cholesterol lowering, antiulcer, hepatoprotective and cardiovascular protective property of the tree. The roots have been reported to possess antispasmodic activity through calcium channel blockade which forms the basis for its traditional use in diarrhoea. It also possesses antimicrobial activity due to its principle component pterygospermin. The fresh leaf juice was found to inhibit the growth of human pathogens as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. (Luqman *et al.*, 2011). Flowers are used as stimulant, tonic and

diuretic. They are useful in increasing the flow of bile. The seeds of *Moringa* are considered to be antipyretic, acrid, bitter and reported to show antimicrobial activity (Tak and Maurya, 2015).