Review of Literature

2.0 REVIEW OF LITERATURE

The review of literature deals with the study **"Evaluation of thrombolytic and antioxidant potential of *Piper betle L.*"** are discussed under the following objectives:

2.1 Medicinal plants

2.2 Phytocomponents

2.3 Free radicals

2.4 Oxidative stress

2.5 Antioxidants

2.5.1 Enzymic antioxidants

2.5.2 Non-enzymic antioxidants

2.6 Thrombolysis

2.7 Cytotoxic activity

2.8 Docking studies

2.9 Plant selected for the study

2.1 MEDICINAL PLANTS

The definition of Medicinal Plant has been formulated by WHO (World Health Organization) as follows- "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants" (Bahar *et al.*, 2013). Medicinal plants are the most exclusine sources of life saving drugs for the majority of the world's population. The medicinal actions of plants are unique to a particular plant species, consistent with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct (Balasubramanian *et al*., 2012).

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions. It is also gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality of life (Behera *et al*., 2012).

Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties (Kavit and Patel, 2013). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The medicinal plants are rich in secondary metabolites which are potential sources of drugs. It has been reported the free radical scavenging and antimicrobial activity of many medicinal plants are responsible for therapeutic effects against cancer, inflammatory, cardiovascular and infectious diseases (Rajamurugan *et al*., 2013).

2.2 PHYTOCOMPONENTS

Plants produce wide array of bioactive principles and constitute a rich source of medicines. In many developing countries, traditional medicine is one of the primary health care systems. Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is therefore necessary. Isolation and characterization of the bioactive molecules ultimately lead to new drug development (Islam *et al*., 2013). Phytochemicals also provide characteristic colour, aroma and flavour in plants. In humans, many phytochemicals have been found to be protective and preventive against many degenerative diseases and pathological processes such as in ageing, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts, and inflammation (Ijeomah *et al.*, 2012).

Phytochemicals are responsible for medicinal activity of plants and they have protected human from various diseases. The major constituents of phytochemical consist of carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and among others. The phytochemical constituents are playing a significant role in the identification of crude drugs. There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects (Maobe *et al.*, 2013).

2.3 FREE RADICALS

Free radicals play a vital role in most major health problems like cancer, rheumatoid arthritis, cardiovascular diseases, Alzeihmer's disease and other neurodegenerative disorders. Antioxidant that scavenges these free radicals proves to be beneficial for these disorders as they prevent damage against cell proteins, lipids and carbohydrates. Antioxidant activity includes free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity (Chakraborty *et al.*, 2011).

Reactive oxygen species (ROS) are highly reactive chemical species with an unpaired electron and formed by catalyzing transition metals like iron, copper, or manganese. ROS are formed in oxidative processes that normally occur at relatively low levels in all cells and tissues. Under normal conditions, the concentrations of ROS are kept under strict control by the activity of a complex defense system including enzymes and non- enzymatic species. In contrast, high doses and/or inadequate removal of ROS results in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (Sakarya *et al.*, 2011).

ROS are the most likely agents inducing DNA damage in atherosclerosis. It generally exists in all aerobic cells in balance with biochemical antioxidants. Recently, increased oxidative stress and impaired antioxidant defence have been suggested as contributory factors for initiation and progression of complications in coronary artery diseases. DNA damage has been found as an emerging risk factor to play an important role in atherosclerosis and coronary artery disease and is caused by multiple endogenous and exogenous factors such as oxidative stress, age, smoking, hypertension, hyperlipidemia and diabetes mellitus. Such damages, if left unrepaired, can cause mutations, which can lead to disease (Simon *et al.*, 2013).

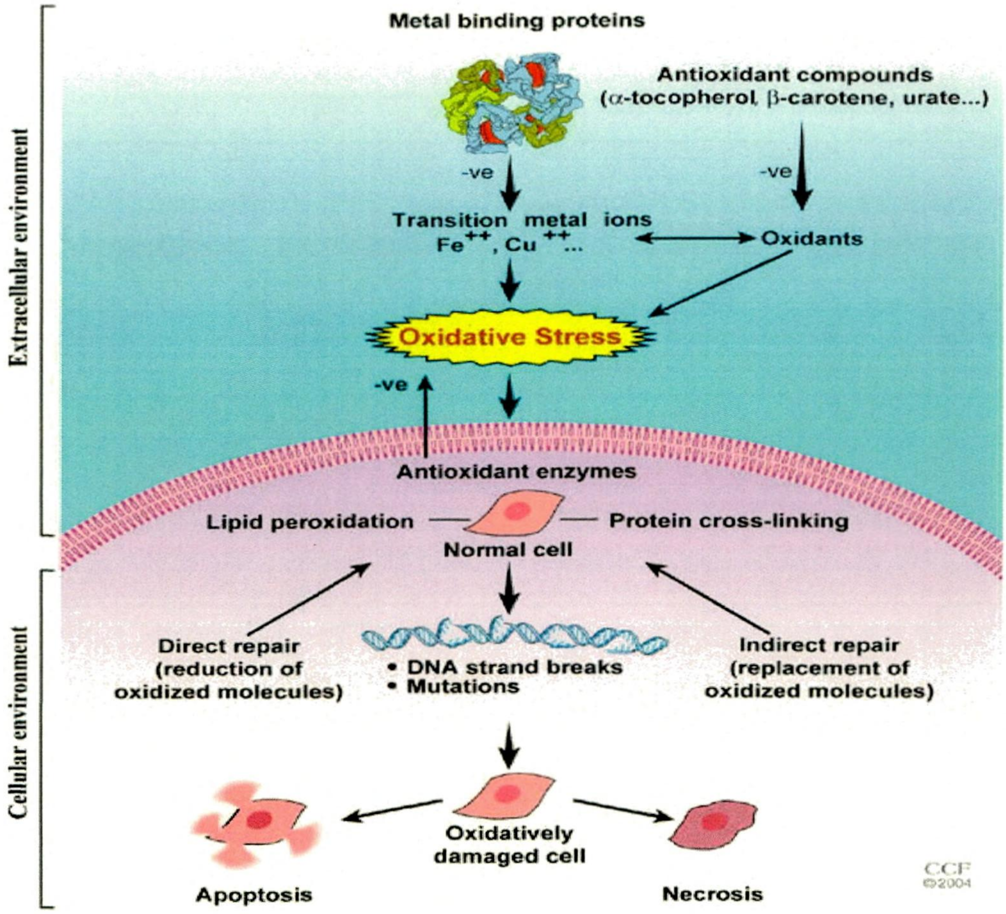
2.4 OXIDATIVE STRESS

Oxidative stress is a leading cause to damage cells by oxidation. Oxidation reactions can involve the production of free radicals which can form dangerous chain reactions. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves. Antioxidants are often reducing agents such as thiols or phenols. Therefore, we need antioxidants to ensure our defence mechanism for neutralizing harmful radicals (Saha *et al.*, 2011).

Oxidative stress results from increased ROS and/or reactive nitrogen species (RNS).Examples of ROS include charged species such as superoxide and the hydroxyl radical, and uncharged species such as hydrogen peroxide and singlet oxygen (Matough *et al.*, 2012). Non-enzymatic antioxidants albumin, uric acid, bilirubin, vitamin C and vitamin E act in concert to reduce the oxidative damage by scavenging free radicals and by detoxifying the oxidants. Malondialdehyde (MDA) the end product of lipid peroxidation by ROS is used as the biomarker of oxidative stress (Shrestha *et al.*, 2012).

FIGURE I

Oxidative stress



2.5 ANTIOXIDANTS

Antioxidant compounds are free radical scavengers because they inhibit or delay the oxidation of substrate by free radicals thereby resulting in significant prevention of lipid peroxidation in biological systems. Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages. These compounds, including flavonols, quercetin, catechins and anthocyanins, exhibit similar structural chemistry whose functions among others is to strengthen the oxidative stability of foods and human systems due to their redox properties, which can play significant roles in neutralizing free radicals, quenching singlet oxygen or decomposing hydroperoxides (Aliyu *et al.*, 2013).

The body has developed several endogenous antioxidant defense systems classified into two groups such as enzymatic and non enzymatic. The enzymatic defense system includes different endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non enzymatic defense system includes vitamin E, vitamin C and reduced glutathione (GSH). Non-enzymatic antioxidants also can be divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants are the endogenous antioxidants, which produced by metabolism in the body like glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferring etc. Nutrient antioxidants are exogenous antioxidants, which cannot be produced in the body but provided through diet or supplements viz. trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids etc (Patil *et al.*, 2012).

The use of synthetic antioxidants such as Butylated Hydroxytoluene (BHT), tannic acid and propylgallate has been reported to be harmful to human health. Hence, strong restrictions have been placed on their application and the trend now is to substitute them with naturally occurring antioxidants (Eleazu *et al.*, 2013).In recent years, several dietary and herbal formulations that have free radical scavenging potential have gained attention in treating such chronic diseases. Inspite of the strong radical scavenging activity of synthetic antioxidant, they usually have side effects thus the interest in finding natural antioxidants, without undesirable side effects, has increased greatly. The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of human diseases. Recently, the residues or byproducts of agriculture industry take attention for their valuable source of natural antioxidants (Sayed *et al.*, 2012).

Such investigations have shown that the positive treatment effects and antioxidant properties of medicinal plants could be correlated with bioactive components such as alkaloids, flavonoids, bioflavonoids, glycosides, mucilage, saponins, tannins, phenols, phenolic acids, coumarins, terpenoids, essential oils, lectins and polypeptides . The use of these compounds as natural antioxidants plays an important role in protecting and prevention of DNA damage, cancer, atherosclerosis and the aging processes (Mazandarani *et al.*, 2013).

2.5.1 ENZYMIC ANTIOXIDANTS

Enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds (Kumar *et al.*, 2011).

Catalase

Catalase is antioxidant enzyme which plays an important role in the body defense mechanism against the harmful effects of the reactive oxygen species (ROS) and free radicals in biological systems (Gacche *et al.*, 2010).

Superoxide dismutase

Superoxide dismutase (SOD), this is one of the most effective intracellular enzymatic antioxidants and it catalyzes the conversion of superoxide anions to molecular oxygen and hydrogen peroxide. Superoxide dismutase exists in several isoforms, which differ in the nature of active metal centre, amino acid composition, co-factors and other features (Rahman *et al.*, 2007).

Glutathione peroxidase

Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organisms from oxidative damage. The enzyme plays an important role in peroxide detoxification. Glutathione peroxides utilize the reducing equivalents of glutathione to reduce hydrogen peroxide and it may be the main mechanism for protection against the deleterious effects of hydroperoxides (Gomathi *et al.*, 2012).

2.5.2. NON-ENZYMIC ANTIOXIDANTS

Non-enzymatic antioxidants such as glutathione, vitamin E (α–tocopherol), vitamin A (retinol), vitamin C (ascorbic acid) carotenoids, thioredoxin, lipoic acid, and ubiquinone act in concert to protect against the free radical- induced damage, mutagenesis and carcinogenesis (Halliwell *et al*., 2007).

Vitamin C

Vitamin C is the non enzymatic antioxidant exists within normal cells as well as they can be supplied through diet. It reacts with free radicals to form radicals themselves which are less reactive than the radicals. They break radical chain reactions by trapping peroxyl and other reactive radicals (Patil *et al*., 2012).

Vitamin E

Vitamin E (α-tocopherol) is probably the most important lipid- soluble antioxidant protecting membranes, lipids and lipoproteins. Vitamin E is one of the few nutrients for which supplementation with higher than recommended levels have been shown to enhance immune response and resistance to diseases. Many studies have suggested that high intake of Vitamin E may slow down the development and progression of atherosclerosis. Some clinical trials also reported beneficial effects of Vitamin E supplementation in the secondary prevention of cardiovascular events (Selvi *et al*., 2007).

Reduced glutathione

Reduced glutathione (GSH) is a tripeptide, non-enzymatic biological antioxidant present in the liver. It protects cellular proteins against reactive oxygen species generated from exposure to CCl_4 . The ability of plant extracts to reactivate the hepatic glutathione reductase was reflected by decreasing the level of lipid peroxidation (Bhandarkar and Khan, 2004).

2.6 THROMBOLYSIS

Medicinal plant extracts demonstrated that they can lyse thrombus as streptokinase. Medicinal plants play a leading role in the treatment of varieties of human diseases from the dusk of human development. Thrombolysis is the breakdown (lysis) of blood clots by pharmacological mean. It is colloquially referred to as clot busting for the reason. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator. Thrombolysis requires the use of thrombolytic drugs, which are either derived from Streptokinase species. Some commonly used thrombolytics are: Streptokinase, Urokinase, Reteplase, and Tenecteplase. Formation of blood clot lies at the basis of a number of serious diseases. By breaking down the clot, the disease process can be arrested, or the complication reduced. While other anticoagulants (such as heparin) decrease the growth of a clot, thrombolytic agent actively reduces the size of the clot. Diseases where thrombolysis is used: Myocardial infarction, Stroke (ischemic stroke), Massive pulmonary embolism, Acute limb ischaemia (Rishikesh *et al.*, 2013).

More selective thrombin inhibitors and antiplatelet agents are more potent, but their safety remains to be confirmed. Continued investigation in this area will provide new insights and promote progress toward the development of the ideal thrombolytic therapy, characterized by maximized stable coronary arterial thrombolysis with minimal bleeding. Several third generation thrombolytic agents have been developed. Compared with the second generation agents (altreplase), third generation thrombolytic agents such as monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase, and staphylokinase result in a greater angiographic potency rate in patients with acute myocardial infarction, although, thus far, mortality rates have been similar for those few drugs that have been studied in large - scale trials. Bleeding risk, however, may be greater. Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase was one example, which has been reported to enhance fibrinolytic activity in plasma and the production of tPA (Elumalai *et al.*, 2012).

2.7 CYTOTOXIC ACTIVITY

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal, etc. 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. Thus, *in vivo* lethality of an extract against a simple zoological organism (brine shrimp napulii) can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products (Chakma *et al.*, 2013).

The Brine shrimp lethality test is simple, portable, reliable, rapidly conducted and its results significantly correlate with the state of toxicity and therefore, BSLT is a reliable answer to routine requests for toxicity screening (Mirzaei *et al.*, 2013). *Artemia salina* the brine shrimp is an invertebrate component of the fauna of saline aquatic and marine ecosystem. It plays an important role in the energy flow of the food chain. It can be used in the laboratory bioassay in order to determine the toxicity by the estimation of medium lethality concentration LC_{50}, which has reported for a series of toxins and plant extracts (Ramachandran *et al.*, 2011).

2.8 DOCKING STUDIES

Molecular docking plays an important role in the rational design of drugs. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking can be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. It was then employed for the analysis with training set composed synthesized compound whose inhibitory activity is unknown, in order to find out the molecular facilities responsible for biological activities (Jubie *et al.*, 2012).

Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and a receptor, fit together and docks to each other well. The molecules binding to a receptor inhibit its function and thus act as drug. The collection of drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations (Sivakumar *et al.*, 2011).

The coagulation cascade of hemostasis has two pathways, the contact activation pathway (formerly known as the intrinsic pathway), and the tissue factor pathway (formerly known as the extrinsic pathway), which lead to fibrin formation. It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the tissue factor pathway (Tanaka *et al.*, 2009). The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by roman numerals, with a lowercase 'a' appended to indicate an active form. The coagulation factors are generally serine proteases (enzymes). The main role of the tissue factor pathway is to generate a "thrombin burst," a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released instantaneously. Anti-coagulation factor VIIa (FVIIa) circulates in a higher amount than any other activated coagulation factor (Johri *et al.*, 2011).

2.9 PLANT SELECTED FOR THE STUDY

Kingdom	: Plantae
(unranked)	: Angiospermae
(unranked)	: Magnoliidae
Order	: Piperales
Family	: Piperaceae
Genus	: *Piper*
Species	: *P. betle*

Binomial name : *Piper betle L.*

PLATE I

Piper betle L.

Piper betle Linn., (family Piperaceae) commonly known as the betel vine is an important medicinal and recreational plant in Southeast Asia. The most probable place of origin of betel vine is Malaysia but today the plants are also cultivated in India, Srilanka, Bangladesh, Burma and Nepal. Based on the color, size, taste and aroma there are many varieties of betel leaf and some of the most popular Indian varieties are the Magadhi, Venmony, Mysore, Salem, Calcutta, Banarasi, Kauri, Ghanagete and Bagerhati (Shah *et al.*, 2013).

The sugars identified in betel leaves include glucose, fructose, maltose and sucrose. The average content of free reducing sugars in different types of betel leaves varies from 0.38-1.46%. It also contains the enzyme like diastase and catalase. It was also reported that the leaves contain vitamins and significant amounts of all the essential amino acids except lysine, histidine and arginine which occur in traces. The leaves used as antiseptic, to stop excessive bleeding during mensturation, stimulant, to relieve throat pain, carminative, expectorant. The leaf juice is used for fever, cough, fatigue, asthma and to disinfect wounds externally (Periyanayagam *et al.*, 2012).

Hydroxychavicol (1-allyl-3, 4-dihydroxybenzene), a catecholic compound present in the aqueous extract of the Piper betle leaf (family: Piperaceae) which is extensively consumed as betel quid in the Indian subcontinent. The compound is better known for its antioxidant and anticancer properties (Hemamalini *et al.*, 2012). Another bioactive phytochemical found in betel leaves are eugenol (EU) which contribute to the beneficial bioactivity. IUPAC name of HC is 3, 4 EU is 3-methoxy-4-hydroxyallylbenzene hydroxychavicol and eugenol (Tee *et al.,* 2012).