Chapter 4

Pharmacological Screening

Pharmacology
Redefining drug discovery and drug research
4. PHARMACOLOGICAL SCREENING OF *FICUS GLOMERATA* ROXB

INTRODUCTION

Pharmacology

The study of drugs and their effects on biological reactions of living beings is called Pharmacology. Pharmacology being an elementary science it has launched itself to the leading phase of contemporary medicine. Success of pharmacology is confirmed by its well established treatment to cure diseases to save lives. A billion dollar turnover flows towards international pharmaceutical industry by pharmacology field.

Pharmacology and its subdivisions

The interaction of chemical compounds with organism and their tissues and cells are related to chemical pharmacology which is a biomedical science. In particular chemical pharmacology is related to the expression of disease and mechanism of drugs against them and their effect on fertility. The methods of synthesis of drugs or isolation of bioactive principles or preparation of pharmaceutical products are basically not spotlighted in the field of pharmacology. Pharmacokinetics and pharmaco dynamics are two main subclasses of pharmacology. The disciplines that deal with these subjects are described below.

Pharmacokinetics deals with the determination of drug concentration in body fluids, tissues at a particular period, which also includes ADME studies i.e. absorption, distribution, metabolism (biotransformation) and excretion of drug.

The study of action of drugs on target organs is referred as pharmacodynamics. In brief pharmacodynamics is the response of drug to body and pharmacokinetics is the response of body towards the drug.
Current pharmacology focuses the physiological effects of drugs by biochemical and molecular mechanisms and with the dose-response relationship, which is nothing but the relationship between the drug concentration in a tissue and the level of the tissue’s response to that particular drug. Most of the drugs effect on target tissues by binding to its protein receptors and this series of mechanisms is referred as signal transduction.

**Toxicology**

The study of poisonous substances and organ toxicity is known as toxicology. It deals with the mechanism of drugs and other chemicals effect, and the mechanisms of toxicants which produce pathologic changes, disease, and death. Like pharmacology, toxicology is also involves the relationship between the dose of drug and the biological effects produced by toxicants on specific tissues. At adequate level of doses, most of the drugs have toxic effects and may have undesirable effects related to toxicity at remedial doses.

**Drug sources and preparations**

A natural product, chemical compound, or pharmaceutical preparation proposed to diagnose or to treat a disease of human or animal, when administered to them is called as drug. The word drug is originated from the French word drogue, means dried herbs and same word also applies to herbs used for cooking in market place rather than any medicinal region. Medicaments and medications are similar words having same meaning as drugs. Dried herb marijuana medical use is strongly discussed in many societies today.

**Natural sources of drugs**

Plants, microbes, animal tissues, and minerals have been the important sources of drug. Alkaloids are the nitrogen containing substances, which gives an alkaline
reaction in alkaline solution among the various types of drugs derived from plants. Morphine, cocaine, atropine, and quinine are few examples for alkaloids. Numerous microorganisms are used as a source of antibiotics. Penicillin and Streptomycin are the two important antibiotics isolated from Penicillium and Streptomyces species. Animals synthesizes the hormones which are acting as most common type of drug, lithium a drug which is used to treat bipolar mental illness and few other therapeutic agents were obtained by mineral sources.

**Synthetic drugs**

In the 19th century modern chemistry facilitated scientists to synthesize new compounds and to alter naturally occurring drugs. Some of the drugs to be synthesized in laboratory for the first are aspirin, barbiturates, and local anesthetics (e.g., procaine). Naturally occurring compound’s, semi synthetic derivatives are led to discovery of new drugs with different properties, for example morphine derivative oxycodone.

Active screening of large number of complex molecules for a specific pharmacologic activity and their use as drug for another purpose apart from its original function has lead to discovery of specific kind of drug to particular diseases accidently. Researchers from medicinal chemistry are utilizing the bioinformatics software for molecular modelling. It helps to detect the structure-activity relationship, between the drug molecule and its target receptor and their pharmacologic activity. Virtual models of drug molecules which fit at their best with three dimensional confirmation of the receptor are created using virtual methods and are synthesized. In designing and synthesizing of drugs or agents that inhibit angio-tension synthesis, for curing hypertension, and inhibition of maturation of the human immunodeficiency virus in AIDS patients can be done by virtual methods.
Drug preparations

Drug preparations include crude drug formulation obtained from natural sources, pure compounds isolated from natural sources or synthesized in the laboratory, and pharmaceutical preparations of drugs intended for administration to patients.

Crude drug preparations

Drying and pulverizing methods can be used for preparation of some crude drugs from plant or animal tissue. Other methods are extraction of bioactive principles from natural products using hot or cold solvent systems. Alcohol and its derivatives can be used as solvents for extraction. Preparations of coffee and tea are familiar examples which are made from distillates of *Coffea arabica* and *Camellia sinensis* plants beans and leaves.

Pure drug compounds

Due to multiple ingredients and varying quantity from batch to batch makes difficulties to identify and quantify the pharmacologic effects of crude drug preparations. In context of growth of pharmaceutical and rational therapeutics, the development of methods to isolate pure form of drugs or compounds from natural sources is an important step. First pure form of natural drug was isolated from a German based company, Frederick Serturner (Susan, 2007).

Phytochemicals and pharmacology

Several researchers have suggested various mode of action of phytochemicals. Inhibition of micro organisms by the action of phytochemicals may be, by interfering with some metabolic processes or by modulate gene expression and signal transduction pathways (Kris-Etherton et al., 2002; Manson 2003; Surh, 2003). Chemotherapeutic and chemo preventive nature of phytochemicals may be used as in
Phytochemical and Pharmacological Profiling of Ficus glomerata Roxb

Chemoprevention. Chemoprevention is use of chemical substances/compounds to regulate the process of tumour tumour generation. Hence, phytochemicals having chemo preventive properties are appropriate to treat cancer, keeping the view that both chemoprevention as well as cancer therapy involves similar mechanism at molecular level. (D’Incalci et al., 2005; Sarkar and Li, 2006).

The general mechanism of action of phytochemicals against microbes is believed to be the cytoplasmic membrane disturbance, proton motive force disruption, electron flow, active transport, and coagulation of cell contents. Mode of actions against bacterial strains by phytochemicals and essential oils involves increasing the permeability of cells by interfering with the phospholipids bilayer of the cell membrane, which results in the leakage of cellular constituency. Breaking the enzyme complexes or their pathways to reduce cellular energy, breakdown or inactivation of genetic material of the cell and causing damage to the cell structural components and cytoskeleton. (Kotzekidou et al., 2008; Doughari, 2012).

**Antimicrobial properties of phytochemicals**

Pathogenic bacteria are the causes of numerous clinical problems across the world. Infectious diseases caused by pathogens are responsible for increased health costs as well as high morbidity and mortality, particularly in developing countries (Roja and Rao, 2007). The increase in the prevalence of multiple drug resistance is necessitated the search for new antimicrobials from alternative or natural sources (Kubmarawa et al., 2007). One way to prevent the clinical problems arising from infection of pathogens is by using new compounds, which are un-related to the existing synthetic antimicrobial agents (Cowan, 1990). Phytochemicals from medicinal plants showing antimicrobial activities have the potential components of filling this need because their structures are different from those of the more studied
microbial sources. Natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to human (Djipa et al., 2000). According to the World Health Organization’s statement; the traditional healing provides the primary health care needs for a large section (80%) of the population (Collins et al., 1995). In India Unani, siddha and Homeopathy prescriptions constitute about 95% of traditional based medicines (Andrews 2001; Londonkar and Kirankumar, 2013).

**Historic view antimicrobials from plant origin**

Human health and well-being is blessed with novel drugs derived from medicinal plants or their parts and these drugs inspired man to search more and more potent bioactive agents for his health care. Plant derived drugs helps in two ways. One is by acting as basic information for the development of a medicine. Second one is treatment of diseases by direct use of phytomedicinal formulations. History provides number of illustrations for drugs of plant origin. To mention few as anti-infective, are the treatment of amoebosis and Entamoeba histolytica infections which causes abscesses by spreading were been treated from many years using isoquinoline alkaloid emetine obtained from the underground part of Cephaelis ipecacuanha and related species.

Alkaloid compound quinine has a long history as an important drug of plant origin. This quinine is present in the bark of Cinchona tree and it occurs naturally. Important medicinal property of quinine is to cure malaria; apart from it can be also used to relieve nocturnal leg cramps. As some strains of malarial parasites have become resistant to the quinines, therefore newer anti malarial drugs with novel mode of action are required. At present chloroquine an analogue of quinine is the widely prescribed drug for treatment of malaria. Higher plants have made remarkable
contributions in the area of anti infectives and beyond them, including anticancer drugs obtained from the Madagascar periwinkle *Catharanthus roseus* such as antileukaemic alkaloids, vinblastine and vincristine (Nelson, 1982).

**Antimicrobials from plants a present context**

The most important advantage of usage of drugs from medicinal plants are comparatively safer than alternative synthetic drugs with and More than 50% of western drugs contain phyto constituents or they have provided basis for synthesis new synthetic or semi synthetic drugs (Robbers et al., 1996). The drugs of modern era with reported biological activities were initially used in crude form by the ancient phyto therapists in traditional or folk remedial treatments. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering reasonable treatment and intense therapeutic benefits.

**Restorative benefit**

Usage and exploration of natural products for treatment of microbial infections arises from microbial sources. Successful search of penicillin provided a path with clear vision towards the discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin. Even higher plants, fungi and other soil micro organisms have been potent source of clinically preferred antibiotics. Bacteriostatic and antifungicidal properties of Lichens, antibiotic action of allinine isolated from *Allium sativum* and berberines from *Hydrastis canadensis* are some examples to mention (Trease and Evans, 1972).

**Economic benefit**

Developing the plant based medicines into potential medicines is a boon in perspective of phytomedicine and drug development. There is a renewed interest in natural products throughout the globe. This eye grabbing attention of people from
synthetic drugs to herbal drugs is due to following factors: People believe that natural products are superior than commercially available drugs; Dissatisfaction of consumer’s with conventional medicines and frequent changes in laws allowing structure-function claims which results in more liberal advertising; aging baby boomers; national concerns for health care cost. The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines. A market of herbal products provides an immediate source of financial benefit to plants based antimicrobials (Ciocan, 2007).

**Antioxidants and phytochemicals**

Most of the biological systems that evolved to live require molecular oxygen for their life; hence oxygen is an essential element to the living beings. Oxygen has variety of prominent roles in many biological Oxidative properties of oxygen play a vital role in diverse biological reactions. Apart from playing as an essential molecule role in all biological reactions; it can also aggravate the damage within the cell by oxidative events as a double edged sword. (Sen et al., 2010).

Any molecule or its molecular components having imbalanced outer atomic or molecular orbits which can exist independently are called free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are depict free radicals and other non-radical reactive by products. The radicals are stable for lesser time than non radical species but in general their reactivity is stronger than non-radical species though radicals are less stable. Homolytic chemical bond breakdown of a molecule occurring via redox reactions results in formation of highly reactive free radicals, once formed these radicals can initiate a series of reaction (Sen et al., 2010).
Electrons are generally found to be connected in pairs in the structure of atoms and molecules. In an atomic or molecular orbit each pair of electrons moves in a specific region of space called orbit.

The term free radical is used due to the independent existing capability of any radical species as alone in an orbital. The orbit may consist one unpaired electrons or more unpaired electrons. An atom of the element hydrogen is the simplest form of free radical which has one proton and one electron. Few examples for unpaired electron is located on oxygen, i.e, oxygen-related radicals are superoxide (O$_2$.2) and hydroxyl (OH.). Sulfur centred radicals are thyl radicals, Carbon centered radical is trichloromethyl (CCl$_3$.) Free radical in which an unpaired electron is displaced between two different atoms is a nitric oxide (NO.) centered free radical (Halliwell, 2001).

**Free radical's sources and their generation**

Free radicals formation in cells and environment is a continuous process. They can be produced from either endogenic substances or exogenic substances. Different sources of free radicals are as follows:

**Exogenous formation**

- UV radiations, X-rays, gamma rays and microwave radiation.
- Metal-catalyzed reactions.
- Oxygen free radicals in the atmosphere are considered as pollutants.
- Industrial effluents, excess chemicals, alcoholic intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins and xenobiotics.
- Interaction with chemicals, automobile exhaust fumes, smoking of cigarettes, cigars, beedie.
- Burning of organic matter during cooking, forest fires, volcanic activities etc.
Endogenous formation

- Inflammation initiates neutrophils and macrophages to produce ROS and RNS.
- Neutrophils stimulated by exposure to microbes.
- In mitochondria-catalyzed electron transport reactions, oxygen free radicals produced as by product.
- ROS formed from several sources like mitochondrial cytochrome oxidase, xanthine oxidases, neutrophils and by lipid peroxidation.
- ROS generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells (Sen et al., 2001).

Oxidative stress

The situation of severe imbalance between production of reactive species and antioxidant defence refers to oxidative stress. First time in the year 1985 Sies has introduced the term oxidative stress in the title of the book he edited. Later in 1991 he has defined the oxidative stress in the introduction of the second edition of the book as a disturbance in the pro oxidant–antioxidant balance, leading to potential damage’.

Effects of oxidative stress on human body was described by Halliwell (2001) as below and stress may produce following consequences:

1. Adaptation: Up regulation of antioxidant defense systems. He has explained with experimental result and observed that, when elevated oxygen is given to adult rats they get acclimatized to the high level of oxygen. Tolerances towards pure form of oxygen by rats become for much longer period when compare to control group rats. This is due to the increased synthesis of antioxidant defense enzyme and Gluta thione sulphatase.
2. **Tissue injury:** DNA, Protein and Lipids are molecular targets of oxidative stress. Agents causing oxidative stress can damage these biomolecules. It is not clearly understood that which molecule is the first point of attack, since injury mechanisms overlie generally. Cellular targets of oxidative stress is based on the tissue selected for study, the primary cellular target of oxidative stress can vary. Addition of \( \text{H}_2\text{O}_2 \) to the mammalian cells increases DNA strand breakage which occurs before the lipid peroxidation or oxidative protein damage detection. Conversion of \( \text{H}_2\text{O}_2 \) into OH reaction in the nucleus with transition metal ions causes DNA damage.

3. **Cell death:** Two mechanisms of cell death are necrosis and apoptosis. Oxidative stress can cause both of these two reactions. Swelling and rupturing of cell swells and releases its contents into the surrounding area and affect the adjacent cells, these are the symptoms of necrotic cell death. Contents can include antioxidants such as catalase or glutathione reductase, and prooxidants like copper and iron ions. Necrotic cell death by the biological mechanisms can also lead to oxidative stress in the surrounding environment of cells. Apoptosis of a cell involves activation of its own internal ‘suicide mechanism’; Hence, apoptosing cells do not release their contents and do not cause any harm to neighboring cells. Certain diseases like neurodegenerative illness involves accelerated cell death by means of apoptosis and in these cases oxidative stress effect has been concerned (Halliwell, 2001).

**Carbon tetrachloride induced hepatotoxicity**

Administration of \( \text{CCl}_4 \) to rats is an accepted experimental model to produce hepatic and other tissue damage due to the presence of fundamental structural similarity between rats and humans.
Carbon tetrachloride (CCl₄) is a lipid-soluble potent hepatotoxic agent when it bound to lipid and protein produces peroxidative degeneration of many tissues. Release of hepatic enzymes (e.g., transaminases, lactate dehydrogenase, sorbitol dehydrogenase), destruction of cytochrome P-450, hepatocellular necrosis, and lipid peroxidation products such as malondialdehyde and 4-hydroxynonenol was observed at increasing level significantly when CCl₄ administered to animals. Metabolism of carbon tetra chloride to trichloromethyl radical decides the level of toxicity of CCl₄. The trichloromethyl radical (CCl₃), in the presence of oxygen interacts with it to form the more toxic compound CCl₃O₂ (Melin, 2000; Hartley, 1999).

The interaction of reactive radicals of CCl₄ with unsaturated fatty acids of membrane lipids initiates the lipid peroxidation. Increased free radical production due to CCl₄ intoxication results in oxidative stress which in turn plays an important role in the degenerative processes in the tissues. Ingestion of CCl₄ is rather rare, but this toxic agent is able to induce tissue damage. It is acceptably reported that the toxic effect of CCl₄ on liver and kidneys can be modulated by the treatment of antioxidants such as vitamins C and E or S-adenosyl methionine. The possibility of treatment of acute CCl₄ poisoning created interest in antioxidant enzymes (Stanislawa, 2003).

**Antioxidants**

Any substance that delay or inhibit oxidative damage to a target molecule are called as antioxidants. A carbon stealing reaction can be ended by the action of one antioxidant molecule. Single antioxidant molecule at a time can react with single free radicals and by donating one of their own electrons it neutralizes free radicals. Scavenging nature of antioxidants may prevent cell and tissue damage. Protection against free radicals can be managed by cell through preventative mechanisms; physical defenses repair mechanisms, and antioxidant defenses (Jacob, 1995).
A variety of components act against free radicals to neutralize them from both endogenous and exogenous in origin.

These include:

- Endogenous enzymatic antioxidants.
- Non enzymatic, metabolic and nutrient antioxidants.
- Metal binding proteins like ferritin, lactoferrin, albumin and ceruloplasmin. Phytoconstituents and phytonutrients.

Endogenous antioxidants produced by body neutralize free radicals and protects tissue injury which results in various diseases. Exogenous antioxidants play an important role to protect the body which can be obtained through food externally (Sen et al., 2010).

Production of oxygen free radicals in the biological system, leads to the functional and structural damages to the cell. Free radicals are the key factors in causing cancer, aging, immuno suppression, inflammation, ischemic heart disease and neurodegenerative disorders (Halliwall, 1994; Gey, 1990). In biological system, antioxidants such as phenolic compounds and vitamins are involved in promoting health also prevent aging and chronic diseases (Larson, 1988).

Phenolic compounds synthesized primarily from products of the Shikimic acid pathway, have several important roles in plants (Shahidi and Nackzack, 1995). Ethno medical literature contains a large number of plants those can be used against free radical mediated diseases (Nig et al., 2000). The undesirable effects of synthetic antioxidants lead to their limited use in treating human diseases. Hence, there is much attraction drawn towards the natural antioxidants derived from plant origin, which could protect cellular machinery from free radicals (Farr, 1997).
Due to the diversity of chemical components with varied structure and synergetic effects among different antioxidants are found in food; it is difficult to relay on a single antioxidant assay to estimate the antioxidant capacity (Hill, 1979). Several invitro assays have been frequently followed to estimate antioxidant capacities in fresh fruits and vegetables and their products for clinical studies. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2 azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), NBTS, Metal chelating activity (MCA) and total antioxidant assays are said to be more relevant because, they utilizes biologically relevant radical source (Andressa et al., 2013). Significant correlation regression exist between total phenolics and antioxidant activity supports the antioxidant potentiality of the bioactive drug (Londonkar and Kirankumar, 2014).

Imbalance in the production and detoxification of free radicals by the biological system may cause oxidative stress in living organisms. The oxidative stress in biological system results in the generation of free radicals or reactive oxygen species (ROS) like superoxide, hydroxyl and peroxyl (Niki, 2004) The unstable free radicals are mediators of inflammation through this they interact with platelets, granulocytes which are also involved in production and activation of ecosinoids to release the cytokines, which brings the oxidative stress by spreading from one organ to other (Recknagel, 1985).

An antioxidant is a molecule capable of lowering or preventing the oxidation of other molecules (Cao et al., 1996) The most common inbuilt reactive oxygen species (ROS) of biological system include superoxide anion, hydrogen peroxide (H$_2$O$_2$), peroxyl radicals, reactive hydroxyl (OH-) radicals and nitrogen derived free radicals (Slater 1965). Production of high amount of reactive oxygen species can
interfere with the inbuilt antioxidant system and induces damages to the cells, tissues and organs (Gerhard, 2002).

Hence, there is a need to develop novel drugs from traditional medicine to protect and strengthen the biological system to avoid serious illness of mankind. Now a days the liver, cardiac and cancer diseases are more predominant in human population and there is lack of satisfactory hepato protective and anti oxidant drugs are available in the market, this made to explore the medicinal plants for the search of potent drug which has less or no side effect and more potent to cure the deadly diseases (Recknagel et al., 1989; Ram and Goel, 1989; Londonkar and Kirankumar, 2014).

**Clot lysis properties of phytochemicals**

Coagulation is the process in which the body prevents blood loss by the formation of a blood clot (thrombus) that stops further blood loss from damaged tissues, blood vessels or organs. Coagulation is a complicated process with a cellular system contains platelets and action of multiple proteins. Platelets circulate in the blood and serve to form a platelet plug over damaged vessels and clotting factors (multiple proteins) act together to form a fibrin clot. Failure of platelets action or clotting factor action leads to disorders in which excessive clotting or very less clotting of blood occurs.

**Factors that accelerate clot formation**

Factor V and factor VIII accelerate the conversion of factor X to factor Xa by factor IXa (this is done by factor VIII) and accelerate the conversion of prothrombin to thrombin as done by factor Xa.

**Factors that inhibit (slow down) clot formation**
Protein C, protein S and thrombo modulin form a complex (group of proteins) that can inactivate factor VIII and factor V. The protein C, protein S and thrombo modulin complex is activated by thrombin (Gurupreet and Shibba, 2013).

**Thrombolysis or fibrinolysis**

The mechanism of fibrin– clot formation physiology is relatively well understood. A blood clot or thrombus consists of blood cells occluded in a matrix of the protein fibrin. Dissolution of the fibrin clot by enzyme-mediated reaction is known as thrombolysis or fibrinolysis. Enzyme plasmin, a trypsin-like serine protease is responsible for fibrinolysis in mammalian blood circulation system. The inactive protein plasminogen present in serum fibrinolytically get converts to active plasmin (Castellino, 1984).

The process of conversion of inactive plasminogen to active fibrinolytic plasmin involves a restricted Proteolytic cleavage by the mediation of different activators of plasminogen. Tissue type plasminogen activators (tPA) and urokinase type plasminogen activators (uPA) are naturally occurring form of plasminogen activators in blood. Plasminogen activator inhibitor-1, PAI-1, a fast-acting inhibitor of tPA and uPA and plasmin α1-antiplasmin, α2 macroglobulin are the general inhibitors of plasminogen activators to initiate fibrinolytic activity in circulating system (Francis and Marder, 1991). Dissolution of fibrin clot involves a series of reactions.

Present clinical interventions are using recombinant normal human plasminogen activators tPA and uPA. A bacterial protein Streptokinase (sPA) is a bacterial protein which is also commonly used plasminogen activator. It does not occur naturally in human circulation. Tissue type plasminogen activator and urokinase type plasminogen activators and streptokinase are having an indirect action in fibrinolytic activity and their restorative action is by converting inactive blood
plasminogen to active form of clot dissolving plasmin. The plasminogen activating action of streptokinase is fundamentally different from the proteolytic activation brought about by tPA and uPA, tPA and uPA, are proteases which possesses their own enzymatic activity but streptokinase do not possesses enzymatic activity of its own. The Proteolytic and plasminogen activating properties to streptokinase is gained by joining with circulatory plasminogen. This combination results in high-affinity streptokinase –plasminogen activator complex, i.e., a stoichiometric complex. The so formed stoichiometric complex proteolytically activates other plasminogen molecules to plasmin by its high-specificity. Hence, the streptokinase role in plasminogen activation is fundamentally different from the proteolytic activation carried out by tPA and uPA. (Banarjee et al., 2004).

History of thrombolytic agents

Streptokinase was discovered by Dr William Tillett in the year 1933. He made an observation that the bacterial strain streptococci agglutinates plasma but not serum, through this observation he has made a conclusion that any plasma containing streptococci would not clot and this laid the foundation for thrombolysis in various settings. The term ‘streptokinase’ was coined by Christensen and MacLeod in 1945. Patients suffering from tuberculosis hemorrhagic pleural effusions and tuberculous meningitis are treated with streptokinase. In 1958, Fletcher first reported the use of thrombolytic therapy for the management of acute myocardial infections. This breakthrough discovery of streptokinase in the treatment of patients with acute myocardial infections was followed by a search for model clot lysis agent, which led to the emergence of second generation and third generation thrombolytic drugs.
Streptokinase

Streptokinase is an extracellular enzyme produced by different strains of h-hemolytic streptococci. The enzyme commission number of streptokinase is EC 3.4.99.22. It is a single chain polypeptide protein capable of activating circulatory plasminogen resulting in thrombolysis. In 1982, Jackson and Tang established the complete amino acid sequence of streptokinase. Streptokinase has a molar mass of 47 kDa and is made up of 414 amino acid residues. The protein exhibits its maximum activity at a pH of approximately 7.5 and its isoelectric pH is 4.7. It is a simple protein and hence, does not contain phosphorous, conjugated carbohydrates and lipids. Structures of Streptokinases produced by different groups of streptococci are differ considerably (Huang et al., 1989; Malke, 1993).

Biophysical techniques such a circular dichroism, nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FT–IR) spectros-copy and differentially scanning calorimetry (DSC) have presented most of the available structural information on streptokinase to the scientific community. A significant level of heterogeneity is present among the streptokinase produced by different categories of streptococci. Fragmental studies of streptokinase have also provided valuable information (Kim et al., 1996).

Alpha, beta and gamma are multiple structural domains of streptokinase—with differentially associated functional properties. Presence of two different domains in streptokinase is suggested by scanning calorimetric analysis. The N-terminal domain has amino acid residues from 1–59 and it has been found to complement the low plasminogen activation ability of the 60–414 amino acid residue domain of the protein streptokinase. (Nihalani et al., 1998; Banarjee, 2004).

Role of natural products in clot lysis
The mechanism of thrombolytic drugs (tPA, streptokinase (SK), and uPA) have different mechanisms to effectively dissolve blood clots; they differ in their detailed mechanisms in ways that alter their selectivity for fibrin clots. The streptokinase has efficiency to bind equally to plasminogen of circulating and non-circulating system, results in significant fibrinogen lysis along with clot fibrinolysis (Naderi et al., 2005). Because of this reason, tissue type plasminogen activators are generally preferred as a thrombolytic agent over streprokinases, especially in treatment of coronary and cerebral vascular thrombosis problems.

Streptokinase is derived from streptococci; patients who have had recent streptococci infections can require significantly higher doses of streptokinase to produce thrombolysis. It is used in patients who have undergone surgery or those with a history of nervous lesions, gastrointestinal bleeding or hypertension (Naderi et al., 2005).

The treatment with tissue type plasminogen activator is restricted in platelet-rich thrombi which are highly resistant to lysis by t-PA. Significant attempts have been continued towards the discovery and development of drugs from products from various plant sources which have antiplatelet, anticoagulant and thrombolytic activity. (Eduardo, 2014).

In prevention and treatment of human diseases plant derived medicines have a well established history. Recent advances in phytochemistry and isolation, identification of plant compounds to heal diseases have rehabilitated the interest in herbal medicines; Effectiveness and site specific nature of the plant based drugs can be brought by modification with recombinant drugs. Even they may be incorporated as a thrombolytic agent for the treatment of the patients suffering from atherothrombotic diseases (Kowalski et al., 2009).
Several side effects of commercially available thrombolytic drugs have been reported and these effects sometimes led to the death of patients due to bleeding and embolism. In this regard, on the basis of the beneficial effects of clot dissolving properties of plant extracts or their products, these agents should be considered as a complement to substitute for thrombolytic agents. (Capstick and Henry 2005; Eduardo, 2014).

**Antimitotic properties of phytochemicals**

Any chemical compounds which arrest cells in mitosis are referred as antimitotic agents. Vinca alkaloids vinblastine, vincristine, and vinorelbine and the taxanes paclitaxel and docetaxel are some of the clinically important anticancer. By interfering with the assembly or disassembly of alpha and beta tubulin into microtubules these antimitotic drugs cause delay in mitosis. Vinca alkaloids and most of other anti mitotics at high concentrations, cause complete microtubule depolymerisation, on the other hand taxanes cause bundling of microtubules by stabilizing them against depolymerisation (Rowinsky, 1997).

Though there was neither depolymerisation nor bundling was seen at low concentrations, but there is sufficient alteration in the tubulin dynamics loss or addition at the ends of mitotic spindle microtubules to prevent the spindle from carrying out its function of attaching to and segregating the chromosomes, and cells arrest in mitosis (Jordan, 1993).

These drugs have valuable role but they are not ideal due to their toxicities. More essentially, several cancers are inherently resistant to these drugs or develop resistance during long time treatment. The over expression of P-glycoprotein which functions as a drug efflux pump results in multidrug resistance. Increases expression of tubulin isotypes, alteration in alpha and beta tubulin structure by mutation or
posttranslational modification reduces tubulin binding efficacy of drugs resulting in drug resistance (Rowinsky and Donehower, 1996).

By serendipity many antimitotic drugs have been discovered, on basis of patterns of toxicity shown by them against different cell lines. Few of them have shown appreciable results in preclinical studies and entered to clinical phase trials. Antimitotic compounds having different chemical structures may exhibit high level specificity to mitotic microtubules than neuronal microtubules and by decreasing unwanted adverse effects. The search for improved antimitotics would be intensely supported by rational assays for screening drugs (Jordan et al., 1998, Paul et al., 1992).

*A. cepa* root tip assay was first introduced by Levan and further it was anticipated as a standard method for studying genotoxicity; and for studying mitotic index depression as reported by previous studies. Colchicine alkaloids vincristine and vinblastine were previously have been accounted for metaphase arrest. Colchicine prevents polymerization of binding tubulin by replacement of a methyl group. (Khanna, 2013).

The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drugs with antimitotic activity (Auti et al., 2010; Angayarakani et al., 2007). The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and after this it even extends a few mm. Cells of this region undergo repeated divisions, and the fate of cell division is higher in this region compared to that of the other tissues; hence, this region is called the meristematic region (Jordan, 2002). This rapid division of meristematic cells is similar to that of the cancer cell division in humans. Hence, these meristematic cells can be used for preliminary
screening of drugs with anticancer activity.

Though the doubts can be raised about extrapolation of results from plant tissue to animals and finally to humans, plant cells are 1000 times more resistant to colchicines which are a potent anticarcinogen and act by inhibiting the microtubule formation. It is inferred that the chemicals which affect plant chromosomes will also affect animals (Saxena et al., 2005; Londonkar and Kirankumar, 2014).
MATERIALS AND METHODS

Antibacterial assay

Chemicals and Microbial Cultures

All the solvents (analytical grade) used in this experimental work are purchased from Merck, Germany. Standard Antibiotics and Culture media - Mueller-Hinton agar (MHA), Nutrient broth (NB), purchased from Hi-Media Laboratories Mumbai. Standard bacterial cultures of *Escherichia coli* (MTCC 46), *Staphylococcus aureus* (MTCC 96), *Salmonella typhimurium* (MTCC 98), *Enterobacter aerogenes* (MTCC 111) and *Klebsiella pneumoniae* (MTCC 432) were procured from IMTECH, Chandigarh, India.

Well Diffusion Method

The antibacterial potencies of chloroform, methanol and aqueous extracts are screened using agar-well diffusion method (Londonkar et al., 2013). The bacterial isolates standardized to 0.5 McFarland in nutrient broth for 18 hr. 100μl of the standardized cell suspension was spreaded on a Mueller-Hinton agar. Wells are punched using a sterile 6 mm cork borer. 50 μl of the crude extract (50 mg/ml) was added into the wells, allowed to stand at room temperature for 1 hr and then incubated at 37°C for 24hrs. The plates were observed for zone of inhibition after incubation period. The effects of various extracts are compared with standard chemotherapeutic agent streptomycin sulphate (50 mg/ml). 1% Tween 80 was used as vehicle to dissolve the extracts.

Minimum Inhibitory Concentration

Minimum inhibitory concentration of the extracts was determined in Nutrient broth by micro-dilution method according to the National Committee for Clinical Laboratory Standards (WHO 2000). Standardized inoculums (0.1 ml, 106 cfu/ml) and
the extracts in different concentrations of 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/ml are taken in test tubes. Test tubes with positive control (Streptomycin), negative control (Methanol) and samples (extracts) are incubated at 37°C for 18 h. The lowest concentration that produced no visible bacterial growth was compared with the control tubes and regarded as Minimum inhibitory concentration.

**Antimitotic and clot lysis activity**

**Chemicals**

Streptokinase lyophilized (Solonase, 15,00,000I.U. Cadila Health Care Limited) was purchased from local medical store, vincristine sulphate was purchased from Sigma Aldrich, and phosphate buffer saline, methanol, and all other chemicals are purchased from Merck Pvt Ltd.

**Extract Preparation**

Powder of a fruit sample was subjected to extract in soxhlet extraction using petroleum ether, chloroform, and methanol solvents successively. Collected extract was centrifuged at 5,000rpm for 10min and filtered. The filtrate was used to conduct further study.

**Blood sample**

2mL of blood was drawn from tail veins of healthy albino rats, using a standard protocol approved by Institutional Ethical committee of Gulbarga University, Gulbarga. Collected blood was transferred to six eppendorf tubes and allowed to form a clot.

**Allium cepa**

Healthy and fresh onion bulbs weighing 40–50g were purchased from the local market of Gulbarga, Karnataka, India. Onions with green leaves/shooting, moldy, and dried bulbs are discarded, where as healthy, disease free onion bulbs are
In vitro clot lysis activity

Clot lysis activity was carried out as per the method of Prasad et al., (2006). Venous blood drawn from the healthy albino rats was distributed in five different pre weighed sterile micro centrifuge tubes and incubated at 37°C for 45min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube− weight of tube alone). To each micro centrifuge tube containing pre weighed clot, 100 μL of plant extracts was added separately. As a positive control, 100 μL of streptokinase and, as a negative non thrombolytic control, 100 μL of sterile distilled water were separately added to the control tubes. All the tubes are then incubated at 37°C for 90min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the differences in weight after the clot disruption. Difference in weight was taken before and after the clot lysis and it was expressed as percentage of clot lysis. Percentage of clot lysis was calculated using the formula:

\[
\frac{\text{weight of released clot}}{\text{Clot weight}} \times 100
\]

Antimitotic assay in onion root tips

Antimitotic activity of *Ficus glomerata* Roxb fruit extracts was carried out. Dried outer layers of healthy onion bulbs were removed and placed over a series of jars containing normal tap water, until to grow 3-4cm of roots from each bulb and tap water was changed at interval of 24hrs. After the root development, bulbs are considered as viable bulbs and water content was removed using tissue paper and these bulbs were selected for the study. These selected roots are treated with
petroleum ether, chloroform, and methanol extracts where as the positive control is treated with vincristine sulphate. A blank with tap water was used as negative control. After 72hr of treatment, the roots were taken out and total root length and number of roots per bulb are measured and root tips were cut and transferred to fixing solution acetic acid: ethanol in the ratio of 1:3 and slightly warmed in 1N HCl. The treated root tips are then stained with aceticarmine. The slide was observed under microscope to count the number of cells, non dividing and dividing cells. Mitotic index was calculated using the following formula:

\[
\text{Mitotic Index} = \frac{\text{Number of dividing cell}}{\text{Total number of cells}} \times 100
\]

**Invitro antioxidant assay**

**Extraction**

The fruit samples are ground to fine powder and passed through a sieve. The filtered samples were dried at room temperature. 10g of the sample powder was extracted with 100 ml of water at 80\(^\circ\)C for 30 min in a water bath shaker. After cooling, the extract was centrifuged at 5,000 rpm for 10 min and filtered. The filtrate was stored at 40\(^\circ\)C for further use.

**Chemicals**

1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroaceticacid (TAC), ethylene diamine tetraacetic acid (EDTA), potassium ferricyanide, ferricchloride, ferrozine, nitrobluetetrazolium(NBT), phenazine methosulfate(PMS), nicotinamide adenine dinucleotide reduced(NADH), Gallic acid are obtained from HiMedia Laboratories, Mumbai, India. All other Chemicals and Solvents used were of high purity and of analytical grade marketed by Sigma Aldrich, Mumbai, India.
Estimation of total phenolic content

The total phenolic content of the extracts was determined by the Folin Cio calteau method described by (Andressa, 2013). 1gm/10 ml of sample was filtered with whatman no.1 paper. 0.5 ml of the sample was added to 2.5 ml of 0.2 N Folin Cio calteau reagent and placed for 5 minutes. 2 ml of 75 g/l of Na₂CO₃ was added and the then the total volume was made up to 25 ml using distilled water. The above solution was kept for incubation at room temperature for 2 hours. Absorbance was measured at 760 nm using 1 cm cuvette in UV-VIS spectrophotometer. Gallic acid (0 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalents (GAE) / g of extract.

Invitro antioxidant studies

DPPH radical scavenging assay

The free radical scavenging activity of Ficus glomerata Roxb methanolic extracts was measured in vitro by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (Brusal, 2011). 0.3mM solution of DPPH in 80% methanol was prepared and 1ml of this solution was added to 3 ml of the extract dissolved in methanol at different concentrations (2.5-20 mg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min under dark conditions and the absorbance was measured at 517 nm using a spectrophotometer. The percentage of scavenging activity at different concentrations was determined and the IC₅₀ values of the extracts are compared with that of ascorbic acid, which was used as the standard. DPPH radical-scavenging activity is calculated according to the following equation:

\[
\% \text{ Inhibition} = \left( \frac{(A_0 - A_1)}{A_0} \right) \times 100
\]

Where \(A_0\) was the absorbance of the control (without extract) and \(A_1\) was the absorbance in the presence of the extract.
**ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity**

ABTS radical scavenging activity was estimated (Mclarty 1997). The stock solutions include 7 mM ABTS solution and 2.4 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for overnight at room temperature in the dark. The solution was diluted by mixing 1 ml of ABTS solution with 60 ml ethanol. Different concentrations (2.5–20mg) of the extracts (1 ml) are allowed to react with 1 ml of the ABTS solution and the absorbance was measured at 734 nm after 7 min using a UV-Visible Spectrophotometer.

**FRAP (Ferric reducing ability of plasma) assay**

Reducing power of the plant extracts was determined (Liu and Nig 2000). Briefly, different concentrations of extracts (2.5mg/ml– 20mg/ml) are added 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The reaction mixture was allowed to incubate at 500C for 20 min. Then 2.5 ml of trichloroacetic acid (10%) was added to the reaction mixture, which was then centrifuged at 9500 rpm for 10 min. The upper layer of solution (2.5 ml) was recovered and mixed with 2.5 ml distilled water and 2.5 ml FeCl3 (0.1%). The optical density was measured at 700 nm in a spectrophotometer. An increase in the absorbance of reaction mixture indicated the increased reducing power.

**NBT (Nitro blue tetrazolium) superoxide radical scavenging assay**

The scavenging activity of the plant extracts towards superoxide anion radicals was measured (Zang and Wang 2001; Yung 2001). The superoxide anion was generated in 3ml of Tris-HCL buffer (100 mM, pH 7.4) containing 750μl of NADH (936μM) solution and 300μl of different concentrations (10–100μgml−1) of extracts.
L-ascorbic acid was used as positive control. The reaction was initiated by adding 750μl of PMS (120μM) to the mixture. After 5 min of incubation at the room temperature, the absorbance was measured at 560 nm. The percent NBT decolourization of the sample was calculated.

**Total antioxidant activity**

Total antioxidant activity was measured using the method of Rice et al., 1996 and Cai et al., 2004. In this method, different concentration (2.5mg–20 mg/ml) of extract in acetic acid was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction mixture are incubated at 95°C for 90 min and cooled to room temperature. Then the absorbance was measured at 695 nm using single beam UV-visible spectrophotometer against blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

**Correlation Study**

Relationship between phenolic content, radical scavenging and total antioxidant was analyzed using correlation studies.

**Invivo antioxidant activities**

**Chemicals**

Liv- 52 was purchased from Himalaya health care Pvt. Ltd. All other chemicals, CCl4, Olive oil, DMSO, including all solvents used are of high purity and of analytical grade marketed by Hi- Media Laboratories, Mumbai and Sigma Aldrich, Mumbai.

**Experimental Animals**

Healthy Swiss albino rats of wister strain from inbred colony are divided into five groups, each group containing minimum six animals. Animals are maintained
under standard laboratory conditions as fed with standard pellet and water ad libitum described by CFTRI Mysore, India. Animals are allowed free accesses of food and water. Experimental protocols were approved by Institutional Animal ethical committee, Gulbarga University, Gulbarga (1994).

**Acute Toxicity studies**

This study is carried out according to OECD guidelines for testing acute oral toxicity Test No.423 (OECD, 2001). Albino rats are divided into control and test groups and they were fasted overnight prior to the dosing and plant extract was administered orally from 200mg/kg to 4000 mg/kg of body weight. No mortality was seen at the dose of 150mg/kg of body weight and 300mg/kg of body weight. Hence these tested doses are selected for the experimental study.

**Physical observation**

All the animals were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes body weight, food and water consumption. The body weights of each animal were noted once in 5 days and the differences in the body weights were recorded. The amount of extract to be given was calculated based on the body weights of the experimental rats to ensure a constant dose volume/kg body weight of the extract given to the rats. The amount of food and water intake was recorded.

**CCL₄ induced oxidative stress**

Oxidative Stress was induced in test animals by treating with 30% of CCL₄ (2 ml/kg of body weight) intraperitoneally. Group I received single dose of saline at the dose level 2 ml/kg body weight for seven days and served as control. Group II received CCL₄ 2 ml/kg of body weight on first day and last day of dosing, Group III, IV, V received CCL₄ 2 ml/kg of body weight for First day and last day of dosing,
Phytochemical and Pharmacological Profiling of Ficus glomerata Roxb

along with single doses of Liv-52, 150 mg/kg FGME, 300 mg/kg FGME respectively for seven days (Londonkar and Kamble, 2011).

**Drug Administration**

CCl4 was given intraperitoneally and Saline, Liv-52 and plant extracts are given by oral dosing method.

**Group-I:** This group received normal saline.

**Group-II:** This group received 2ml of CCl4 for 7 days from day 1 to day 7 (30% in olive oil).

**Group-III:** This group received 2ml of CCl4 for 7 days from day 1 to day 7 + 2ml of Liv-52 was administered from day 8 to day 14.

**Group -IV:** This group received 2ml of CCl4 for 7 days from day 1 to day 7 + 150mg/kg of FGME was administered from day 8 to day 14.

**Group -V:** This group received 2ml of CCl4 for 7 days from day 1 to day 7 + 300mg/kg of FGME was administered from day 8 to day 14.

On fourteenth day of experiment all animals are sacrificed and blood was collected, in eppendorf tube allowed to clot for 30min and centrifuged at 2500 rpm for 15 min, serum was separated and used for the estimation of biochemical markers. Liver of all animals was dissected out freed from fatty tissue, washed in ice-cold saline, used for histo-pathological studies.

**Estimation of liver marker enzymes**

The serum was used to estimate functional state of liver enzymes and other biochemical markers. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) was estimated using method describe by Abie, (1983).
Estimation of anti–radical enzymes

Liver tissue homogenate was prepared in 0.5 M Tris hydrochloric acid buffer (pH 7.4) at 4°C. The homogenate was centrifuged at 8000rpm for 10min and the supernatant was again spanned at 12000 rpm for 15min, the obtained mitochondrial fractions were used for the assay of enzymes namely Catalase, peroxidase, superoxide dismutase and glutathione reductase (Lawernce and Burk, 1976; Marklund and Marklund, 1974; Lin et al., 1998).

Histopathological Studies

The liver was dissected out and washed in ice-cold saline and fixed in 10% formalin, after 24 hours embedded in paraffin wax.3µ-5µ thin sections were taken with the help of microsection instrument and stained with Haematoxylin and Eosin. Mounted sections are observed and captured under photomicroscope (Lieca). Observation of change in the central vein, sinusoids, hepatocyte, kappa cells and ballooning was studied (Kubsad, 2008; Ward, 1999; Ranawat, 2010).

Statistical Analysis

The results are expressed as the mean ± SEM of six animals in each group. The data was analyzed by one-way ANOVA followed by student’s t-test. The values of P< 0.05 are considered as statistically significant.
RESULTS

Antimicrobial activity

Chloroform, methanol and aqueous extracts of *Ficus glomerata* Roxb fruit was screened for antimicrobial activity using well diffusion assay and minimum inhibition concentration test. Five standard bacterial cultures such as *Escherichia coli* (MTCC 46), *Staphylococcus aureus* (MTCC 96), *Salmonella typhimurium* (MTCC 98), *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumonia* (MTCC 432) are employed for the study.

**Agar well diffusion assay: (Graph 4.1)**

In this assay assessing the values of zone of inhibition by plant extracts, the potency of plant extract was calculated.

Chloroform extract has shown lesser antimicrobial activity by forming marginal zone of inhibition against all micro organisms. Chloroform extract has shown considerable activity by forming 10mm of zone of inhibition against *Staphylococcus aureus* (MTCC 96), followed by 9mm of zone of inhibition against *Salmonella typhimurium* (MTCC 98), and 8mm of zone of inhibition against *Klebsiella pneumonia* (MTCC 432). Very poor activity or negligible zone was formed against *Escherichia coli* (MTCC 46) and *Enterobacter aerogenes* (MTCC 111) i.e, 7mm each.

Methanol extract of *Ficus glomerata* fruits has exhibited highest activity against *Salmonella typhimurium* (MTCC 98) by forming 17mm of zone of inhibition. 16mm of zone of inhibition was recorded against *Escherichia coli* (MTCC 46), 15mm for *Enterobacter aerogenes* (MTCC 111) and average activity was recorded against *Staphylococcus aureus* (MTCC 96) and *Klebsiella pneumonia* (MTCC 432) with the zone of inhibition 10mm and 8mm respectively.
*Ficus glomerata* fruits aqueous extract displayed a significant antimicrobial activity against *Staphylococcus aureus* (MTCC 96) with 17mm of zone of inhibition. Against *Salmonella typhimurium* (MTCC 98) it showed 14mm of zone of inhibition. The growth of *Enterobacter aerogenes* (MTCC 111) and *Staphylococcus aureus* (MTCC 96) was inhibited by forming 10mm and 9mm marginal zone of inhibition respectively. Whereas, least activity was observed against the organism *Klebsiella pneumonia* (MTCC 432) with the formation of 6mm of zone of inhibition.

Standard antibiotic drug streptomycin has shown highest activity against *Enterobacter aerogenes* (MTCC 111) with the zone of inhibition 24mm and It formed 22mm of zone of inhibition against *Escherichia coli* (MTCC 46), *Staphylococcus aureus* (MTCC 96), *Salmonella typhimurium* (MTCC 98) and least activity of standard drug streptomycin was recorded against *Klebsiella pneumonia* (MTCC 432) with 18mm of zone of inhibition.

Negative control distil water has shown nill effect against all micro organisms used in the test.

**Minimum inhibitory concentration assay (Table 4.3)**

Using micro dilution method the lowest concentration of extracts which inhibits the growth of micro organisms was calculated.

*Ficus glomerata* Roxb chloroform extract has shown growth inhibitory activities at the lower concentration 3.12mg/ml each against *Staphylococcus aureus* (MTCC 96), *Enterobacter aerogenes* (MTCC 111) and *Klebsiella pneumonia* (MTCC 432). On the other hand the minimum concentration required to inhibit the activity of *Escherichia coli* (MTCC 46) and *Salmonella typhimurium* (MTCC 98) was found to be each of 6.25mg/ml of fruit extract.
Methanol extract of *Ficus glomerata* Roxb fruit recorded the significant minimum inhibitory concentration value was 0.78mg/ml against *Enterobacter aerogenes* (MTCC 111) and followed by 1.56mg/ml of methanol extract towards the inhibitory activities of *Escherichia coli* (MTCC 46), *Salmonella typhimurium* (MTCC 98) and *Klebsiella pneumonia* (MTCC 432) in each organism. The growth of *Staphylococcus aureus* (MTCC 96) was inhibited at concentration of 3.12mg/ml after *Ficus glomerata* Roxb fruit extract treatment.

*Ficus glomerata* Roxb aqueous extract also inhibited the growth of pathogenic microbes at broad range of concentrations. The least concentration was found to be 0.78mg/ml for *Klebsiella pneumonia* (MTCC 432), 1.56mg/ml against *Enterobacter aerogenes* (MTCC 111) followed by 3.12mg/ml against *Escherichia coli* (MTCC 46) and *Salmonella typhimurium* (MTCC 98). Whereas 6.25mg/ml concentration of fruit extract has inhibited the growth of *Staphylococcus aureus* (MTCC 96).

**In vitro clot lysis activity: (Graph 4.2)**

As a part of discovery of cardio protective drugs from natural resources the different extractives of *Ficus glomerata* Roxb are assessed for thrombolytic activity. In this test, Addition of 100 μl SK (Streptokinase), a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C has showed 74.65 ± 3.606 % lysis of clot. On the other hand, distilled water was treated as negative control which has exhibited a negligible percentages of lysis of clot (4.50 ± 1.110 %). The in vitro thrombolytic activity study revealed that, methanol extract of *Ficus glomerata* Roxb fruits has exhibited highest thrombolytic activity (47.23 ± 2.778%). However, significant thrombolytic activity was demonstrated by the aqueous and chloroform extracts which have shown 27.89 ± 3.418%, and 23.11% ± 2.656%, respectively.
Antimitotic activity (Graph 4.3 and Table 4.4)

It is observed that *Ficus glomerata* Roxb fruit extracts stunted the growth and development of onion roots. In addition, the number of roots and average root length was lesser in that plants which are exposed to fruit extracts than the control groups. Out of three extracts, methanol extract was found highly effective in the reduction of root number and length, followed by chloroform extract and petroleum ether extract as compared to normal control group which is treated with tap water.

Numbers of roots in negative control tap water treated onion bulbs are found to be 13 and average root length was $4.31 \pm 0.23\text{cm}$ and positive control which received vincristine sulphate a standard drug shown 5 roots per onion bulb with average root length $2.19 \pm 0.27\text{cm}$. The test sample *Ficus glomerata* Roxb petroleum ether extract marginally considerably reduced the number of roots to 8 and root length to $3.58 \pm 0.47\text{cm}$. Number of roots in chloroform extracts treated onion bulbs are 11 and average root length was $4.17 \pm 0.13\text{cm}$. Methanol extract of *Ficus glomerata* Roxb fruits significantly reduced the root numbers up to 7 with average root length $3.11 \pm 0.56\text{cm}$ when compared to standard drug vincristine has, which has shown 5 roots per onion bulb and $2.19 \pm 0.27\text{cm}$ root length (Fig 4.7).

The effect of *Ficus glomerata* Roxb fruit extracts on mitotic index of *Allium cepa* root cells was recorded. Petroleum ether extract has shown $66.11\%$ of mitotic index, chloroform extract treated onion roots showed $42.50\%$ of mitotic index, methanol extract considerably reduced the mitotic index value $23.50\%$ when compared to other test groups and standard drug shown mitotic index of $7.90\%$.

**Invitro antioxidant assay**

Total phenolic content, free radical scavenging capacity and total antioxidant assays of methanol extract of *Ficus glomerata* Roxb was estimated using by Folin Cio
Phytochemical and Pharmacological Profiling of Ficus glomerata Roxb

Department of Biotechnology, Gulbarga University, Kalaburagi

Department of Biotechnology, Gulbarga University, Kalaburagi

Phytochemical and Pharmacological Profiling of Ficus glomerata Roxb

Department of Biotechnology, Gulbarga University, Kalaburagi

Department of Biotechnology, Gulbarga University, Kalaburagi

Department of Biotechnology, Gulbarga University, Kalaburagi

calteau method, DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay, ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) assay, FRAP (Ferric reducing ability of plasma) assay, NBT (Nitro blue tetrazolium) superoxide radical scavenging assay.

**Total phenolic content (Graph 4.4)**

The presence of total phenolic content in *Ficus glomerata* fruit extract is reported in Fig.4.8. in concentration dependent manner. The amount of phenolics varied from 76.77µg/GAE to 351.45 µg /GAE in different range of concentrations of extracts (2.5mg/ml-20mg/ml). 76.77/ µg GAE of phenolics are present in 2.5mg/ml of methanol extract of *Ficus glomerata* fruits. At a concentration of 5mg /ml of fruit extract 118.5µg /GAE of phenolics were found. 10mg/ml concentration of methanol extract found to contain 222µg/GAE of phenolics. 351.45µg/GAE of phenolics are present in 20mg/ml concentration of methanol extract of *Ficus glomerata* fruit extract.

**DPPH radical scavenging assay : (Graph 4.5)**

Free radical scavenging ability of methanol extract of *Ficus glomerata* Roxb fruit extract was studied by DDPH assay method. The percentage of inhibition of free radicals by FGME (Ficus glomerata methanol extract) was 27.31%, 41.08%, 65.44% and79.26% at concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml and 20mg/ml of FGME. On the other hand standard drug ascorbic acid has shown 79.15%, 82.76%, 83.04% and 85.97% at concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml and 20mg/ml of *Ficus glomerata* methanol extract respectively.

**ABTS assay (Graph 4.6)**

The methanol extract of Ficus glomerata Roxb has exhibited percentage of chelating ability in concentration dependent manner. 2.5mg/ml concentration of *Ficus glomerata* methanol extract chelated 17.33% of ABTS, 5mg/ml 36.08%, 10mg/ml 60.49% and 20mg/ml 76.11%. The standard drug EDTA chelated the ABTS to
35.57%, 65.22%, 75.31% and 88.94% at concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml and 20mg/ml of *Ficus glomerata* methanol extract respectively.

**FRAP Assay: (Graph 4.7)**

In reducing power assay, the ferric ion reducing ability of plasma by *Ficus glomerata* methanol extract was found 11.43% at 2.5mg/ml, 36.28% at 5mg/ml, 67.19% at 10mg/ml and 69.30% at 20mg/ml. The standard drug BHT has shown 82%, 84.53%, 89.68% and 89.92% at concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml and 20mg/ml.

**NBT Assay: (Graph 4.8)**

Nitro blue tetrazolium superoxide radical scavenging ability of FGME was found to be 17.08% at 2.5mg/ml, 36.495 at 5mg/ml, 60.25% at 10mg/ml and 76.59% at 20mg/ml of FGME. Standard drug EDTA has shown 35.52%, 62.74%, 69.43% and 85.91% at concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml and 20mg/ml.

**Total antioxidant activity assay: (Graph 4.9)**

Total antioxidant activity of FGME was calculated by phospho molybdate assay. 2.5mg/ml concentration of FGME has total antioxidant capacity (TAC) 17.34%, 5mg/ml has shown 44.29%, 10mg/ml has shown 68.12% and 20mg/ml has shown 81.37%. The standard drug ascorbic acid has shown 29.52%, 60.08%, 83.94% and 92.77% at concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml and 20mg/ml respectively.

**Correlation Studies: (Table: 4.5)**

Correlation analysis has indicated positive correlation between total phenolic content and with DPPH (r = 0.93), ABTS (r = 0.99) and total antioxidant capacity (r = 0.98). This analysis also indicates a positive correlation of DPPH with ABTS (r = 0.92) and total antioxidant capacity (r = 0.96); ABTS with total antioxidant capacity (r
The findings suggest the strong involvement of phenolics in the antioxidant activity of FGME.

**Toxicity studies**

The acute toxicity study of *Ficus glomerata* Roxb methanol extract did not show any harmful effect on test animals at test concentrations ranging from 200mg/kg body weight to 4000 mg/kg body weight. Based on this observation the drug concentrations lesser than 1/10th and 1/20th of tested drugs were selected for pharmacological study.

**Physical observation**

Physical observation of the animals treated with *Ficus glomerata* Roxb methanol extract throughout the study indicated no adverse behavioural changes. The tested drug at higher concentrations has not shown any mortalities of the animals. The test animals are shown normal behaviour, sleep, food and water intake. No changes were found in skin colour, eye colour and movement. No mortality was observed in any of the experimental rats.

**Gravimetric study (Table 4.6)**

Experimental animals are observed for their change in body weight, food and water intake. There was no significant change has been observed either in body weight of control group and experimental group. Gradual increase in the body weight of both treated and control group rats was observed, indicating that no side effect has been caused by the treatment extract. Also no considerable change is observed in consumption of food and water.

Average body weights of animals before the treatment of drug was 120.8±0.74g in control group, metanolic extract at the dose of 200mg has shown 120.7 ± 0.67g body weight, at 500mg it is 121.0 ± 0.28g and it was 121.0 ± 0.19g
body weight in 1000 mg/kg group, 121.4 ± 0.05g in 2000 mg/kg group and 124.5 ± 0.91g in 4000 mg/kg group extract treated. On day 5th the body weights of rats was found to be 121.2±0.04g in control group, 121.9 ± 0.33g in 200mg/kg methanol extract treated group, 122.5 ± 1.07g in 500 mg/kg treated group, 122.7 ± 0.3g in 1000 mg/kg treated group, 122.8 ± 0.09g in 2000 mg/kg group and 121 ± 0.11g in 4000 mg/kg treated group. On the 10th day of experiment the control group has shown the body weight about 122.1±0.42g, in 200mg/kg treated group it was 124.7±4.38g, in 500mg/kg treated group it has shown 124.0 ±1.25g, in 1000mg/kg treated group has shown 124.0±0.24g and in 2000mg/kg of treated group it was 122.2±0.05g and in 4000mg/kg treated group the body weight was noticed about 120±0.62. On 14th day the body weight of animals in respective groups was found to be 124.0 ± 0.25g in control group, 126.2 ± 7.16g in 200mg methanol extract treated group, 126.3 ± 1.66g in 500mg methanol extract treated group, 128.0 ± 0.35g in 1000mg methanol extract treated group, 128.3 ± 0.06g in 2000mg methanol extract treated group and 125 ± 0.46g in 4000mg methanol extract treated group.

Invivo antioxidant activity

Estimation of liver marker enzymes (Table 4.7)

Liver marker enzymes such as AST, ALT, ALP and LDH are estimated to observe the hepatoprotective nature of methanol extract of *Ficus glomerata* Roxb in comparison with standard drug Liv-52. The levels of AST, ALT, ALP and LDH in normal control group was found to be that ALT 52.52 IU/dl, AST 73.6 IU/dl, ALP 95.25 IU/dl, and LDH 48.07IU/dl. Significant change in the levels of AST, ALT, ALP and LDH levels of CCl₄ treated groups it has observed and it is 142.08IU/dl for ALT, 126.17 IU/dl for AST, 161.82 IU/dl for ALP and 97.92 IU/dl for LDH. In standard drug Liv – 52 treatment to CCl₄ toxicity induced group it has shown the levels of liver
marker enzymes as ALT 87.57 IU/dl, AST 89.00 IU/dl, ALP 122.31 IU/dl, and LDH 44.85 IU/dl. 150mg/kg dose of Ficus glomerata methanol extract treatment to CCl4 toxicity induced group has shown ALT level as 127.16 IU/dl, AST level as 118.39 IU/dl, ALP level as 150.41 IU/dl and LDH level as 87.03 IU/dl. On the other hand 300mg/kg dose of Ficus glomerata methanol extract treatment to CCl4 toxicity induced group has shown reduction in the liver marker enzyme exhibiting ALT level as 104.95 IU/dl, AST level as 107.02 IU/dl, ALP level as 136.14 IU/dl and LDH level as 56.88 IU/dl.

**Estimation of antiradical enzymes**

Level of antiradical enzymes GSH, CAT, POD and SOD in normal control group rats was found to be 20.09 nmol/10mg for GSH, 61.44 U/mg for CAT, 15.28 U/mg for POD and 31.72 U/mg for SOD. In CCl4 treated animals the level of these enzymes indicated about 10.30 nmol/10mg for GSH, 40.10 U/mg for CAT, 9.26 U/mg for POD and 14.17 U/mg for SOD which are highly reduced values when compared to normal rats. But the standard drug Liv – 52 treatment to CCl4 toxicity induced group has shown the antiradical enzyme activities like 18.9 nmol/10mg for GSH, 55.82 U/mg for CAT, 13.01 U/mg for POD and 26.44 U/mg for SOD. The low dose treatment of Ficus glomerata methanol extract at 150mg/kg body weight to CCl4 toxicity induced rats has shown the enzyme GSH values as11.12nmol/10mg, CAT values as 46.17U/mg, POD values as 10.16U/mg and SOD values as 18.03U/mg. The higher dose treatment of Ficus glomerata methanol extract at the dose level 300mg/kg body weight toxicity induced rats has shown the enzyme activity 13.74nmol/10mg for GSH, 50.23U/mg for CAT, 11.54U/mg for POD and 20.97U/mg for SOD respectively.
Histopathological studies (Fig. 4.5)

The histopathological study of liver transverse sections in control rats indicated the presence of normal cellular architecture, with mass of cytoplasm and clearly visible central veins. (Fig. 4.5A), whereas CCl₄ hepatotoxicity induced animals the transverse sections of liver is showing (4.5B) an extensive liver injuries, which is characterized by moderate to severe hepatocellular degeneration and necrosis around the central vein, fatty changes, ballooning degeneration is observed. Liv–52 treatment to CCl₄ hepatotoxicity induced group has showed a significant recovery in the liver structure by observing healthy hepatocytes with distinct central vein and clear cytoplasm(Fig.4.5C) The *Ficus glomerata* Roxb treatment at the lower dose of 150 mg/kg body weight (Fig. 4.5D) to CCl₄ hepatotoxicity induced group has showed a significant recovery in the damage of liver tissue exhibiting protection from hepatocyte degradation, local necrosis and vacuolization of the tissue, while the *Ficus glomerata* methanol extract treatment at high dose of 300mg/kg body weight to CCl₄ hepatotoxicity induced rat group has showed highly significant protection of hepatocyte from degradation, local necrosis, vacuolization (Fig 4.5E) compare to low dose of 150 mg/kg body weight extract treatment this indicates that the recovery of the liver tissue was dose dependent in the *Ficus glomerata* Roxb extract treatment.
DISCUSSION

Antimicrobial activity

Phytochemical screening of medicinal plants is an important process in identifying a new source of therapeutically and industrially important compounds. The antimicrobial activities of phenolics and flavonoids have been studied by many researchers in some plants. Flavonoids are becoming the subject of anti-infective research, and some researchers have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity.

Previous studies of Taha and Abbas (2011), Rahaman et al., (1994), Sen and Chowdhary (2006) indicated the presence of alkaloids, carbohydrates, glycosides, proteins and amino acids, phenolic compounds, flavonoids and terpenoids in some species of plants. The study of Pongothai et al., (2001) on phytochemical screening of Ficus glomerata Roxb bark extract and reports of Jai et al., (2014) on ethanol extract of leaves, fruit and bark of Ficus racemosa Linn also confirms the presence of all the above compounds.

Study of Lee and Jeong (2010) on Ficus carcia leaves extract on methicillin resistant Staphylococcus aureus isolates suggest that the fig leaves could be employed as a natural antibacterial agent in MRSA infection as care products. Where the zone of inhibition was recorded upto 15.6mm and MIC value was 0.08mg/ml proving the efficacy of plant as a source of antimicrobial agent. In the present study all the three extracts of Ficus glomerata Roxb have effectively inhibited the growth of tested bacterial strains. The methanol extract has shown antimicrobial activity with zone of inhibition ranging from 12-20 mm, where as aqueous extracts of Ficus glomerata Roxb was found to have antimicrobial activity with zone of inhibition ranging from 9-17 mm and the chloroform extract has exhibited least activity among three extracts.
with zone of inhibition ranging from 9-12mm. The activity of methanol extract was almost equally and comparable to the standard chemotherapeutic agent streptomycin sulphate zone of inhibition which ranges from 18-24mm. The inhibitory activity of *Ficus glomerata* Roxb extracts against pathogens confirms the potential activity of the plant in the treatment of bacterial diseases. Presence of various chemical compounds in the extract impart significant amount of biological activities of *F. glomerata* Roxb thus proving its medicinal value.

**Antimitotic activity**

The results of the present study indicating that various extracts of *Ficus glomerata* Roxb are having a mild to moderate antimitotic activity. Methanol extract treatment has inhibited the root growth significantly, followed by petroleum ether extract and chloroform extract treatment. This antimitotic activities of the compound was supported by mitotic index and this effect may be possibly due to phytoconstituents present in the plant extracts which are affecting the cytoskeleton or inhibiting the activity of one or more components of the cell cycle, thereby providing clear information regarding cytotoxic action of the fruit extracts as reported by Bhattacharya and Haldar (2010). Growth inhibition effect of the fruit extract may be due to diminished cell division. Therefore, it is evident that the plant extracts are possessing antimitotic activity (Londonkar and Kirankumar, 2014). Onion roots are taken as sampa model for the study of phytotoxic activity as per Williams and Omoh (1996) who have studied on same model with the leaf extract of cymbogon citratus. *Allium cepa* has been used for evaluating cytotoxicity since the early 1920s (Grant, 1988). This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatment as expressed in growth inhibition of the roots of onion bulbs. Levan (1938) has reported that *Allium cepa* test is a rapid, highly sensitive, and
reproducible bioassay for detecting cytotoxicity of phytochemicals and results of Allium test fit well for prokaryotes and other eukaryotes system. Investigations of Nwangburuka and Oyelana (2011) have also showed the lowering of the mitotic index and development of some chromosome abnormalities especially in cells treated with high concentrations of chloroquine. Their study suggests an inhibitory effect of chloroquine on DNA biosynthesis and further this may imply an inhibitory effect of chloroquine on Allium cepa cell growth.

**Clot lysis activity**

Clot lysis may be the result of the combinatorial effect of the active compounds or the individual compounds present in the extract (Prasad et al., 2007). With further research on cell viability tests and in vivo studies, these findings are proved for having important implications of plant extract in the treatment of cardiovascular diseases, which is increasing at an alarming rate. Since the drugs which are used for the cardiovascular diseases are not economical and not accessible to the greater section of the society and they cause side effects, on the contrary the herbal drugs are having less or no side effects and application of phytomedicine may show and be a boon for the above diseased patients.

Study of Mahamud et al., (2013) displays the in vitro thrombolytic potential of crude methanolic extract of *T. crispa* stem using human blood. *T. crispa* stem extracts at two different concentrations (2.5mg/ml - 20mg/ml) exhibited clot lysis activity from average to good (14.81%-25.73%). Standard thrombolytic agent streptokinase dissolved the clot to 50.1%. Rashid et al., (2013) and Elumalai et al., (2012) have screened different plant extracts in search of thrombolytic drug and their results are highly supportive in the use of plant extracts for dissolving clots.
**Invitro antioxidant activity**

The antioxidant activity of plant origin components can be due to the presence of phenolic compounds (Velioglu et al., 1998). Phenolics are aromatic secondary plant metabolites extensively distributed throughout the plant kingdom and associated with colour, sensory qualities, nutritional and antioxidant properties of food (Hang wang et al., 1996). The results of the present experiment indicates that the total phenolic contents of different concentrations of methanol extracts was ranging from, 76.77 mg/GAE to 351.45 mg/GAE. Several researchers have reported the existence of positive linear relationship between antioxidant activity and total phenolic content in many plants and herbs (Rekka and Kourounakis 1991; Shahidi and Naczk 1995; Aruoma and Cuppett, 1994). The antioxidant activity of phenolic compounds is essentially due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers and heavy metal chelators (Shan et al., 2005). Further research is underway to determine specific component which plays the major role. The total antioxidant capacity of *Ficus glomerata* was measured and compared with that of butylated hydroxyl toluene. The results of the above experiment showed that the methanol extract is potential, indicated the concentration dependent antioxidant activities when compared with that of BHT. Reactive oxygen species (ROS) encompass a spectrum of diverse chemical species including superoxide anions, hydrogen peroxide, hydroxyl radicals, nitric oxide and others.

*In vivo* studies results interfaces that oxidants play a variety of role in both animals and plants (Ohsugi, 1999). ROS are involved in cellular signaling, cell growth regulation, specific cellular physiology, and energy production. However, the oxidation of lipids, DNA, protein, and carbohydrate by toxic ROS and cause DNA
mutation also damage target cells or tissues which results in cellular senescence and death (Zi et al., 1997).

Recent, investigations implies that the knowledge and application of antioxidants in reducing oxidative stress at in vivo condition has promoted many investigators to search for potent natural antioxidants from plant sources (Nayan et al., 2011). Various techniques have been used to determine the antioxidant activity through invitro in order to allow rapid screening of substances. In the present study, it is noticed that the methanol extracts of *Ficus glomerata* Roxb fruits exhibited the ability to display antioxidant properties in a dose dependent manner, which is evaluated by various invitro assays.

Radical scavenging activity of *Ficus glomerata* Roxb resolved in the study using various test systems shows that the extract and its active substances could be promising for the further studies. The research on fruits and vegetables indicated the presence of important nutrients and phytochemicals in them which show an antioxidant potentiality (Hamzah et al., 2013).

The above mentioned test systems a comparison study can provide the important information for development of plant extracts and assessment of their antioxidant properties. In the present study in vitro antioxidant tests data reveals that extracts have a poor activity against the formation of hydroxyl radicals, these radicals are harmful radical and they are formed during in vivo studies, but these are more potent in decreasing the concentration of nitrite after the spontaneous decomposition of sodium nitroprusside it indicates that the *Ficus glomerata* Roxb extracts may be able to scavenge nitric oxide. The scavenging effect on DPPH also provides a strong antioxidant effect for the extracts, and this activity may be attributed to their capacity of trapping free radicals by donating a hydrogen atom.
**Invivo antioxidant activities**

Carbon tetrachloride induced hepatotoxicity is the most commonly practiced model system for evaluating hepato protective potency of plant extracts (Reckgenal, 1983).

In the present study, methanolic extract from *Ficus glomerata* fruits are used for the in vivo validation. The hepatotoxic effect of CCl4 is due to its metabolite trichloromethyl radicals, which binds to the biological macromolecules and results in the peroxidative degradation of endoplasmic reticulum membrane (Lin et al., 1998). Increase in the level of SGOT and SGPT in the serum of toxicity induced animals is observed after cellular leakage, and loss of functional integrity of cell membrane it is also due to the free radicals generated by CCl4 (Reckgenal, 1989). Increased levels of SGOT, SGPT, ALP and LDH activity is significantly reduced by the treatment of *Ficus glomerata* methanol extract, when compare to the control in a dose dependent manner, this may be due to the action of phenolics and flavonoids present in the methanol extract of *Ficus glomerata*. This effect may be either by preventing the generation of free radicals or by strengthening hepatocytes activity. Earlier studies on different parts of *Ficus glomerata* have reported the presence of antioxidant flavonoids such as ficusin, rutin and favonoid glycosides (Khan and Javed, 1998; Mandal et al., 1999; Mandal et al., 2000).

Bors et al., (2001) have observed the role of flavonoids in diminishing the effect of superoxide anion by its dismutation and detoxification of free radicals generated by the metabolism of carbon tetra chloride through the coordinated actions of cellular antioxidants. Studies conducted by Niki (2004) and Cao (1996) reveals that the GSH conjugation plays a vital role in detoxification of metabolites, which are the major causative agents liver injury.
Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. Hepatotoxicity induced animals in this experiment has showed decrease in the level of GSH and CAT due to the toxicity of trichloromethyl radicals. Whereas the test animals treated with *Ficus glomerata* methanol extract at the low dose of 150mg/kg of body weight and high dose of 300mg/kg of body weight has showed enhancement in GSH and CAT antioxidants significantly in dose dependent manner.

This was supported by the report of Kubsad et al., (2008), who have reported the role of plant polyphenols in retaining antioxidant status in hepatotoxicity induced animals. Standard drug Liv-52 has also showed an effective increase in the CAT and GSH levels. CCl₄ treatment to rats produces hazardous effects in liver like loss of hepatocytes arrangement, necrosis, and degeneration (Slater, 1965). Treatment of *Ficus glomerata* methanol extract (150mg/kg b/w and 300mg/kg b/w) to the experimental rats has treated groups have retained the normal cellular architecture when compared to standard hepato protective drug Liv-52 treated animals.

Previous studies on Ficus species have shown the presence of antioxidant flavonoids such as ficusin, rutin and favonoid glycosides which are responsible for the repair of tissue damage caused due to CCl₄ treatment (Kengap et al., 2011). Investigations of Mandal et al., (2000) and Hill et al., (1979) also reveals the presence of active compounds in *Ficus glomerata* Roxb for the hepato protective and antioxidant activities. Based on the above observations it can be concluded that the *Ficus glomerata* Roxb is having a potential antioxidant and hepato protective component that can be used as a drug.