Man, ever since his first appearance on Earth, has used plants throughout his historical development as a source of medicines. Plants have formed the basis of the folkloric medicine which was the main source for modern drugs (Newman et al., 2000; El-Ghazali et al., 2010). In the last few decades, there has been an increasing interest in the ethnopharmacological studies of medicinal plants (Dahanukar et al., 2000). Because of this resurgence of interest, the research on the plants of medicinal importance is growing phenomenally at the international level. By the middle of the nineteenth century about 80% of all medicines were derived from plants. Then, due to the scientific revolution in pharmaceutical industry, the synthetic drugs dominated (Gilani and Atta-ur-Rahman, 2005).

1.1. History of medicinal plants

Historically, the use of plants as medicine by mankind for therapeutic purposes dates back to the origin of human civilization (Adewunmi and Ojewole, 2004; Aliyu et al., 2007). Ancient texts of India contain exhaustive depictions of the use of a variety of plant-derived medications (Ahmad et al., 2006). Hippocrates (460 B.C.) was the first Greek to regard medicine as a science; he is popularly known as the father of medicine. His *Materia Medica* consisted herbal recipes as simple remedies for about 400 ailments. *Historian Planetarium* by Theophrastus of Athens (370 B.C.) served as a reference book for ancient medicines. In the 18th century a Swedish naturalist Carolus Linnaeus (revolutionary taxonomist) recorded descriptions of over 5900 plant species, which deal with the identification of plants and their
characteristics, evolutionary histories of plants including catalogues with Latin terminologies (Halberstein, 2005).

Plants have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the 19th century. In the early 20th century, there was a slow erosion of the medicinal plants as sources of therapeutic agents due to the vast usage of synthetic drugs. Later, synthetic drugs are plagued by side-effects and toxicities and this has lead to the re-emergence of the use of medicinal plants (Brater and Walter, 2000). Though plants have served as a major reservoir of new drugs, only a small portion of the (approximately 270,000) known plants have been investigated for its medicinal activity (Allen et al., 2011).

1.2. Ethnomedicinal plants

Medicinal plants have a long-standing history, plays an important role in primary healthcare of traditional societies in many developing countries. Interestingly, one out of four commercial drugs available is plant based. Natural remedies from medicinal plants are found to be safe, effective and techno-economically viable (Bobbarala et al., 2011). The uses of medicinal plants as a source for relief from illness can be traced back over five millennia to the written documents of the early civilization in China, India and the near East, but it is doubtless an art as old as mankind. Herbs provide useful tools for treating various diseases which contain substances that could be used for therapeutic purposes in different countries around the world (Bandyopadhyay et al., 2002; Goleniowski et al., 2006; Abolaji et al., 2007).
According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. Among the estimated (2,500,000) higher plant species on earth, only (35,000 to 70,000 species) less than 1% have been used for medicinal purpose (Ponnu et al., 2003). Plants have served as a broad range of medicinal and pharmaceutical products all over the world. Mostly, the traditional medicines are used by different ethnic groups inhabiting various terrains of developing countries for primary healthcare (Farnsworth et al., 1985; Sandhya et al., 2006). Of late, herbal medicines are in great demand in developed countries, because 25% of drug prescriptions come from natural products (Anyinam, 1995). In 2003, the WHO estimated that, about 80% of people in developing countries rely chiefly on traditional plant based medicines for their primary healthcare (Fairbairn, 1980; Hack-Seang, 2005; Zaidi and Crow, 2005; Gurib-Fakim, 2006; Goyal et al., 2008). People in underdeveloped countries, especially the farmers, rely on wild plants for food, construction materials, fuel wood, medicine and many other purposes. Even today, tribal communities still collect and preserve locally available wild and cultivated plants to treat a number of diseases and disorders (Mahishi et al., 2005). Throughout the ages medicinal plants are used in the treatment and prevention of livestock diseases (Masika and Afolayan, 2002).

As the largest producer of medicinal herbs, India is appropriately called ‘Botanical garden of the world’ (Ahmedulla and Nayar, 1999). Rig-Veda and Atharvana describes about medicinal plants and its uses. About 8000 herbal remedies have been codified in the Ayurveda, which is still in use in many dispensaries even today (Baker et al., 1995; Reddy et al., 2001). In India, medicinal plants are regularly used in various systems of healing because of their wide biological activities,
effectiveness, easy availability, minimal side effect and cost effectiveness (Chopra et al., 1958; Cragg et al., 1997).

1.3. Traditional medicine

The WHO defines traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (WHO, 2002). Traditional medicine is a comprehensive term used to refer both systems such as traditional Chinese medicine and Indian Ayurveda or Arabic Unani medicine. Traditional medicine is often termed ‘complementary’, ‘alternative’ or ‘non-conventional’ medicine (Alves and Rosa, 2007). In many developed countries, complementary and alternative medicine is becoming more and more popular. The use of herbal extracts and nutritional supplements either as alternative or complimentary medicine to the conventional chemotherapy for the treatment of various diseases (Dahanukar et al., 2000). Ethnomedicinal practices serve as an important vehicle for understanding indigenous societies and their relationships with nature (Rai and Lalramnghinglova, 2010).

1.4. Pharmacognostic studies

Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. Quality control of crude drugs is a challenging task because of complex nature of chemical constituents. To ensure the quality of herbal products proper identification of the plant material is essential. According to WHO, the macroscopic and microscopic description of a medicinal
plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (Anonymous, 1996). Correct botanical identity based on the external morphology is possible when a complete plant specimen is available. Anatomical characters can also helps for identification, when the morphological features are indistinct (Cutler et al., 2008).

1.5. Phytochemistry

The term ‘phytochemicals’ refers to a wide variety of chemicals found in plants. Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases (Fukumoto and Mazza, 2000). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also the chemical substances that produce a definite therapeutic action on the human body (Hill, 1952). The phytochemicals do not act alone but most of the time it is in a combination of complexes (Cowan, 1999). Some of the important bioactive compounds are flavonoids, alkaloids, saponins, tannins, glycosides, anthroquinones, sterols, triterpenoids and phenolic compounds.

Flavonoids are hydroxylated polyphenolic compounds widely distributed in plants and the largest group of plant secondary metabolites containing 15 carbon atoms. They are a group of about 4000 naturally occurring compounds known to have contributed to human health through our daily diet (Giulia et al., 1999; Kim et al., 2013). Flavonoids can be classified into five major subgroups, which include: flavones, flavonoids, flavanones, flavanols, anthocyanidins and wide distribution of antibiotic principles (Skinner, 1955; Kuhnau, 1976; Nijveldt et al., 2001). They exhibit antimicrobial and other medicinal properties (Harborne and Williams, 2000).
Medicinal plants contain flavonoid compound possess a high antioxidant potential (Kris-Etherton et al., 2002; Vaya et al., 2003); antimicrobial properties (Harsh et al., 1983; Jit et al., 1986; Kayser and Arndt, 2000) and many biological effects including antiallergic, antiinflammatory, antihapatotoxic, antiulcer, antiviral and antispasmyotic effects (Baez et al., 1999; Xu and Lee, 2001; Ogundipe et al., 2001; Nag et al., 2004). They are efficiently to enhance vase-relaxant process (Bernatova et al., 2002) and prevent platelet activity-related to thrombosis (Wang et al., 2002).

Tannins and tannin like substances are widely distributed in many species of plants. They play an important role in protecting plants from predation and regulating the plant growth. Tannins are bitter and high molecular weight polyphenolic compounds of plant origin with antinutritive effects associated dietary proteins, polymers and minerals thus retarding digestion. Tannins can also impair digestive processes by complexion with enzymes and endogenous proteins (Mc Sweeney et al., 2001). Tannins have stringent properties widely used as an application to sprains, bruises and superficial wounds. They are also possess antidysenteric and antidiarrheal, antimicrobial and antioxidant activities (Rievere et al., 2009) anthelmintic, antiviral and to chelate dietary iron (Prakash et al., 2013) cytotoxic and antineoplastic agents (Aguinaldo et al., 2005).

Essential oils contain highly volatile substances that are extracted from different parts of aromatic crops through hydrodistillation. Volatile (essential) oils are the reservoir of biologically active compounds and are a great benefit to protect humans from pathogens and also to endorse the immune system (Seth et al., 2012). Essential oils are used for preservation of foods, flavouring, antimicrobial, analgesic,
sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies (Baydar et al., 2004; Demirci et al., 2008)

1.6. Pharmacological investigations

The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds for the development of novel and safe medicinal agents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. The phytochemical research based on ethnopharmacological information is generally considered as an effective approach in the discovery of new anti-infective agents (Chhetri et al., 2008).

1.6.1. Anti-haemolytic activity

Haemolysis refers to the destruction of erythrocytes with liberation of haemoglobin in the plasma. Haemolysis occurs in a variety of pathological conditions such as autoimmunity against RBC–surface antigen, mechanical disruption of RBCs (red blood cells), malaria/clostridium infection, thalassemia and sickle cell diseases (Dhaliwal et al., 2004). Erythrocytes are exclusive blood cells that deliver oxygen to our body, act as vendor of nutrients (Giardina et al., 1995) which possess desirable physiological and morphological characteristics and exploited extensively in drug delivery (Hamidi and Tajerzadeh, 2003) and participate in detoxification of a great variety of toxic xenobiotics (Sivilotti, 2004). Furthermore, RBCs are very susceptible to oxidative stress during too high cellular concentration of oxygen and haemoglobin, high polyunsaturated fatty acid content while oxidative stress in RBCs is implicated to haemolysis (Kanti and Syed, 2010). Oxidative damage to the erythrocyte membrane
(lipid/protein) may be implicated in haemolytic associated with some haemoglobinopathies, oxidative drugs, transition metal excess, radiation and deficiencies in some erythrocyte antioxidant systems (Ko et al., 1997).

1.6.2. Antioxidant activity

Free radicals, namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS), are known to cause damage to lipids, proteins, enzymes and nucleic acids leading to cell or tissue injury implicated in the process of ageing. Reactive oxygen species (ROS) are normal by-products of cellular metabolism (Brawek et al., 2010). The production of reactive oxygen species may produce oxidative stress and induce various degenerative diseases (Chen et al., 2010). ROS can be neutralized by antioxidant defense systems including antioxidant enzymes and antioxidant compounds (Lee et al., 2009). Several evidences indicated that oxidative stress can lead to cell and tissue injury. Free radicals, including the superoxide radical (SO\textsuperscript{•−}), hydroxyl radical (OH\textsuperscript{•−}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and lipid peroxide radicals have been implicated in a number of disease processes, including asthma, cancer, cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory diseases, liver diseases, macular degeneration, periodontal, and other inflammatory diseases (Mavi et al., 2003; Mosquera et al., 2007). The therapy using free-radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000).

Plants are known to be the potential sources of natural antioxidants and they contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins, phenolic acids and polyphenols (Lee et al.,
Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombic and vasodilatory activities. Because of their phenolic compounds (Cook and Samman, 1996; Pourmorad et al., 2006). The intake of natural antioxidants from plants have been associated with low incidence of cancer, cardiovascular diseases, diabetes, and other diseases associated with aging (Kao, 1980). Today, there are overwhelming interests in finding naturally occurring antioxidants for use in foods and in medicinal materials to replace synthetic antioxidants (Parr and Bolwell, 2000).

1.6.3. Anti-diabetic activity

Diabetes mellitus is a chronic metabolic disorder and fifth leading cause of dreadful disease which affects 3% of the world population (Pradeepa and Mohan, 2002; Hsu et al., 2008). Inadequate secretion of insulin affects blood glucose concentration (Subbulakshmi and Naik, 2001; Mohan et al., 2004; Hongmei et al., 2005). Diabetes mellitus comprises several heterogeneous group of disorders characterized by hyperglycemia, altered metabolism of carbohydrates, lipids and proteins. It is associated with complications such as nephropathy, retinopathy and cardiovascular disease (Alberti and Zimmette, 1998). Several species of medicinal plants are used in the treatment of diabetes worldwide (Gondwe et al., 2001; Brai et al., 2007). Plants have always been an excellent source of drugs and many of the currently available antidiabetic drugs have been derived directly or indirectly from them (Alarcon-Aguilara et al., 1998; Pradeepa and Mohan, 2002; Tiwari and Rao, 2002). India stands first with largest number of diabetics with 33 million diabetic populations at present and epidemiological studies indicated that the number will rise
to 57 million by the year 2025. It is estimated that the annual cost of diabetic healthcare in India was about Rs.90 billion in an ideal situation of all patients receiving appropriate care (Haffner, 1999).

1.6.4. Anticancer activity

Cancer is a scourge afflicting disease to mankind from the time immemorial. In spite of the spectacular advances made by medical sciences during the present century, the treatment of cancer remains an enigma. It is a major public health burden in both developed and developing countries and the incidence of cancer is increasing annually (Parkin et al., 2005; Shoeb, 2006). Medicinal plants have played an important role in the last half century in the treatment of cancer and most new clinical applications of plant secondary metabolites and their derivatives have been applied towards combating cancer. The antitumor agents are able to kill or inactivate tumor cells without damaging normal tissues. Currently, plants derived anticancer drugs in regular clinical use for the cancer treatment. They are vinblastine and vincristine was isolated from *Catharanthus roseus* (Apocyanaceae) (formerly *Vinca rosea* L.) which is used for the treatment of a variety of cancer, including testicular, breast, lung cancers and Kaposi’s sarcoma (Heijden et al., 2004; Cragg and Newman, 2005). The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (Shoeb, 2006). The screening of some compounds with ten cancer cell lines (A-549, BEL-7402, BGC-823, SGC-7901, DU-145, HT-29, MCF-7, MDA-MB-231, U-251, B-16) where 324 compounds showed cytotoxicity against at least two cancer cell lines (Han et al., 2008). MCF-7 is an estrogen receptor-positive human cancer cell line, which derived from a patient with metastatic breast cancer (Levenson and Jordan, 1997). Growth of
MCF-7 cells is inhibited by tumor necrosis factor (TNF alpha). Many plants are claimed to induce apoptosis in MCF-7 cells such as *Antrodia camphorata* (Yang *et al*., 2006) and *Gmelina asiatica* (Merlin *et al*., 2010).

1.6.5. Larvicidal activity

Vector-borne diseases constitute the major cause of morbidity in most of the tropical and subtropical countries and have always been a challenge to the medical professionals struggling for the welfare of humanity (Hubalek and Halouzka, 1999). Mosquitoes are the major arthropod vectors for the most dreadful and fatal diseases such as dengue, malaria, yellow fever, filariasis, Japanese encephalitis, chikungunya and also cause allergic responses to humans that include local skin and systemic reactions such as angioedema (Peng *et al*., 1999; Das and Ansari, 2003). The vector-borne diseases caused by different species of mosquitoes are *Aedes* sps., *Culex* sps., *Anopheles* sps., etc. which are constitute an unsurpassed health problem all over the world.

In India, malaria is one of the most important causes of direct or indirect infant, child and adult mortality with approximately 2 or 3 million new cases arising each year, is transmitted by mosquitoes (Nagpal and Sharma, 1995; Shell, 1997). The most reliable strategy of minimizing the incidence of vector-borne diseases is in the eradication and management of vectors. One of the methods to control the vectors in order to bring interruption in disease transmission, and the control of mosquitoes in larval stage has been efficient way in the integrated vector management (Rutledge *et al*., 2003). The herbal medicines are used as larvicides, they are efficient and do not harm other organism in the environment (Corbel *et al*., 2004; Rivero *et al*., 2010).
1.6.6. Antimicrobial activity

Bacteria, fungi, viruses and other microorganisms are potentially pathogenic and cause many diseases in humans, animals and plants, even deadly diseases. Bacterial infections are one of the major issues and serious problems in healthcare systems all over the world (Nester et al., 1998). The emergence of bacteria resistance to antibiotics is a severe problem, and cost of treating these infections has become a burden to National Health Service (Samy et al., 2006; Abeysinghe, 2010). Antibiotic resistant bacteria outbreaks have been reported in hospitals throughout the world (Adwan et al., 2008). To develop new compounds with novel mode of action to overcome this problem is required (Gislene et al., 2000; Dewanjee et al., 2007). Medicinal plants represent a rich source of antimicrobial agents (Bhattacharjee et al., 2010; Irudayaraj et al., 2010; Jarrar et al., 2010; Koochak et al., 2010; Raja et al., 2010). Because of the side effects and the resistance of pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts the biologically active compounds from plant species (Kilani and Nagarajan, 2006).

In plants, secondary metabolites are main source of antimicrobial agents (Mahesh and Satish, 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and pharmaceuticals agents. These compounds can be isolated and used to replace and increasing resistance of microbes to synthetic antimicrobials used in both the health and the farming sector (Eloff, 1998; Elgorashi et al., 2004; Shad and Leyva, 2008).
1.7. Need for the present study

The role of natural products in the development of drugs used in modern medicine is unsurpassed even when synthetic chemistry has been developed beyond expectations. The phytochemical and pharmacological investigations have been added to the use of medicinal plants by revealing the presence of active principles and their actions in human and animal systems. Investigations in the field of phytochemical and pharmacology have supplied valuable information on medicinal plants with regard to their biological properties and therapeutic uses.

The various species of plants including the members of the family Verbenaceae are widely and abundantly distributed worldwide. They exhibit various biological and pharmacological properties (Roy and Choudhar, 1983; Nyiligira et al., 2008). *Gmelina asiatica* Linn. is one such medicinal plant used to cure various ailments among the traditional and indigenous people of India since ages (Kiritikar and Basu, 1975; 1984 and 1991). The natural abundance of *G. asiatica* and its potential application in traditional medicine lead to the investigation of its phytochemistry and bioactivity. In the present study, the leaf extracts of *G. asiatica* were screened for phytochemical and bioactive properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the plant.

1.8. Objectives and Plan of Work

On the basis of the above facts and information, the present work has been designed and planned to evolve the strategy for the identification of bioactive compounds from the leaves of *Gmelina asiatica* Linn. against pathogenic organisms
to promote human and animal health. To achieve the aim, the objectives listed below will be addressed.

**Objectives**

- To investigate the anatomical characteristics of *Gmelina asiatica* leaf and stem to establish its pharmacognostical standards.
- To analyse the phytochemical constituents of *Gmelina asiatica* leaf extracts for the establishment of different classes of compounds.
- To evaluate the antimicrobial properties of the leaf extracts of *G. asiatica* against some human pathogenic organisms.
- To determine the *in vitro* antioxidant activities of *G. asiatica* leaf extracts by different radical scavenging methods.
- To find out the functional groups of *G. asiatica* leaf by using Fourier Transform Infrared Spectroscopy (FT-IR).
- To identify the minor as well as major components of the ethanolic leaf extract and essential oil isolated from the leaves of *Gmelina asiatica* by using Gas Chromatography-Mass Spectrometric analysis (GC-MS).
- To evaluate the anticancer and anti-diabetic activity of *Gmelina asiatica* leaf extracts.
- To study the mosquito larvicidal activity of *G. asiatica* leaf extract.

**Plan of Work**

The research work would be carried out according to a well planned and systematic scheme. The steps in systematic work plan are as under.

**Literature review and Selection of plants**

A large number of plants used traditionally are still unexplored. Similarly a large number of widely distributed plants need to be explored for therapeutic utility.
The first step is to review the literature for such potential plant and select such a plant for a systematic study.

**Collection and Authentification of Plants**

The plants selected and the parts of plant decided would be collected from their natural habitat in healthy flourishing form. Sample of plant and/or its parts would be submitted to the Botanical Survey of India, Coimbatore for authentification. After authentification, the parts of plant would be collected in sufficient quantity so as to suffice the requirement of work.

**Pharmacognostic investigations of plant**

The plant parts selected would be investigated for anatomical features.

**Successive Solvent extraction**

The plant parts selected would be extracted with solvents of different polarity to study the phytoconstituents present in them. The plant parts would be extracted with petroleum ether (40-60), chloroform, ethanol and acetone successively.

**Phytochemical investigations of plant extracts**

The extracts obtained would be subjected to phytochemical investigation for the presence of various classes of compounds present in plants. Tests for alkaloids, sterols, saponins, tannins, flavonoids, glycosides, terpenoids, quinones, coumarins, carbohydrates, proteins and phytosterals would be performed on the extracts to detect their presence.
**FT-IR and GC-MS study of plant extracts**

The plant part selected would be subjected to FT-IR spectral analysis and GC-MS analysis for identification of functional groups and major and minor phytoconstituents respectively.

**Pharmacological screening of plant extracts**

The plant extracts would be subjected to determination of pharmacological activity.

The extracts would be subjected tests for following pharmacological studies:

I. *In-vitro* activities
   a. Antibacterial activity
   b. Antioxidant activity
   c. Antidiabetic activity
   d. Antihemolytic activity
   e. Cytotoxic study

II. *In-vivo* activities
   a. Larvicidal bioassay