V. Discussion
The objective of medicine is to address people’s unavoidable needs for emotional and physical healing. The discipline has evolved over millennia by drawing on the religious beliefs and social structures of numerous indigenous peoples, by exploiting natural products in their environments, and more recently by developing and validating therapeutic and preventive approaches using the scientific method. With globalization, the pattern of disease in developing countries is changing. Unlike in the past, when communicable diseases dominated, now 50 percent of the health burden in developing nations is due to non communicable diseases, such as cardiovascular diseases, diabetes, hypertension, depression, and use of tobacco and other addictive substances. Because of lifestyle, diet, obesity, lack of exercise, and stress are important contributing factors in the causation of these non communicable diseases, Complementary and Alternative Medicine (CAM) and Traditional Medicine (TM) approaches to these factors in particular will be increasingly important for the development of future health care strategies for the developing world (Jamison et al. 2006).

One of the phenomena of the last three decades has been the huge increase in use of ‘herbal products’. These can be defined as plants, parts of plants or extracts from plants that are used in healthcare or in combating disease (Mukherjee and Houghton, 2009a). Ethnomedicine may be defined broadly as the use of plants by humans as medicines where as traditional medicine is a broad term used to define any non-Western medical practice. The terms *complementary* and *alternative* describe practices and products that people choose as adjuncts to or as alternatives to western medical approaches. Increasingly, the terms CAM and TM are being used interchangeably (Kaptchuk and Eisenberg, 2001). Ethnopharmacology is a highly (Newman, 2007) diversified approach to drug discovery involving the observation, description, and experimental investigation of indigenous drugs and their biologic activities. It is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines (anthropology,
archaeology, history and linguistics) that contribute to the discovery of natural products with biologic activity.

In recent times, there have been increased waves of interest in the field of research in natural products chemistry. This level of interest can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural products as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, such as chromatographic techniques-TLC, column chromatography HPTLC, HPLC and GC etc. are invaluable for identifying and isolation of phytopharmaceuticals. Adsorption chromatography has proved particularly important in the isolation and purification of vitamins, hormones, many alkaloids, cardiac glycosides anthraquinones etc. Further, use of physical techniques to establish structures of new compounds and to identify known compounds in plant sources ultraviolet, infrared, mass and nuclear magnetic resonance spectroscopy together with X-ray crystallographic and optical rotatory dispersion methods have all played a significant role in these developments. Various modifications of mass spectrometry (MS) have become of increasing importance for the structural characterization and determination of the active constituents of plants (Trease and Evans, 2005). These improved techniques to isolate, purify, and structurally characterize active constituents (Soumya, 2009) are contributing significantly in solving the demand for supply of complex natural products (Clark, 1996). The R & D thrust in the pharmaceutical sector is focused on development of new innovative/indigenous plant based drugs through investigation of leads from the traditional system of medicine (Patwardhan, 2004).

It is somewhat ironic that this ‘return to nature’, as far as medicinal substances are concerned, has occurred at a time when medicine has become increasingly technologically sophisticated, both in the equipment and products
used for diagnosis and treatment, and also in the design and research into the mechanisms underlying disease. Several different reasons have been put forward for the resurgence of interest in and use of herbal products. These include a reaction against the serious side-effects sometimes observed when orthodox drugs are used, especially the more potent ones; the inability of western medicine to treat some diseases satisfactorily, especially chronic conditions such as eczema and arthritis, and the generally mistaken idea that ‘natural’ must be better or safe (Mukherjee and Houghton, 2009b).

Herbal medicinal products occupy a significant place in consumer consciousness in the developed world and are important in healthcare in most developing countries. The value of natural products in this regard can be accessed from: (i) the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semisynthetic and total synthetic modification, (ii) the number of diseases treated or prevented by these substances, and (iii) their frequency of use in the treatment of disease. The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products. Analysis of the sources of new and approved drugs during the period 1981 to 2002 reveals that natural products play a highly significant role in the drug discovery and development process (Jones, 2006). Review of all approved agents during the time frame of more than 25 years from 1981 to 2006 for all diseases worldwide and from 1950 (earliest so far identified) to 2006 for all approved antitumor drugs worldwide reveals the utility of natural products as sources of novel structures, but not necessarily the final drug entity, is still alive and well (Newman, 2007). It is often noted that 25% of all drugs prescribed today come from plants (Farnsworth, 1976 and Raskin and Ripoll, 2004). This estimate suggests that plant-derived drugs make up a significant segment of natural product based pharmaceuticals. Out of many families of secondary metabolites, or compounds on which the growth of a plant is
not dependent, nitrogen-containing alkaloids have contributed the largest number of drugs to the modern pharmacopoeia, ranging in effects from anticholinergics to analgesics and from antiparasitics to anticholinesterases to antineoplastics (Raskin, 2002). Although not as plentiful as alkaloids in the modern pharmacopoeia, terpenoids (including steroids) have made an equally important contribution to human health. They range from Na+/K+ pump-inhibiting cardiac glycosides (Dewick, 2001), to antineoplastic (Cragg, 1998) to antimalarial (Abdin, 2003), to anti-inflammatory (Goldbach-Mansky, 2006 and Kupchan, 1972). The goals of using plants as sources of therapeutic agents are a) to isolate bioactive compounds for direct use as drugs, b) to produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity, c) to use agents as pharmacologic tools and d) to use the whole plant or part of it as a herbal remedy.

Despite several problems, one cannot discount the past importance of plants as sources of structurally novel drugs and it provides a great opportunity to the scientists in the field of Natural Product Chemistry, Pharmacognosy, Pharmacology, Ethnobotany and other related fields of life science to come together and work in the direction of getting new drugs from natural sources, especially from plants for betterment of mankind. Nature is the best combinatorial chemist and till now natural products compounds discovered from medicinal plants (and their analogues thereof) have provided numerous clinically useful drugs. In spite of the various challenges encountered in the medicinal plant based drug discovery, natural products isolated from plants will still remain an essential component in the search for new medicines. In this regard, the results of the pharmacognostic, phytochemical and pharmacological experiments carried out utilizing the plant viz. Anogeissus latifolia are discussed in the proceeding pages.
1. PHARMACOGNOSY

The definition and practice of pharmacognosy have been evolving since the term was first introduced about 200 years ago (Kinghorn, 2001 and Samuelsson, 2004), as drug use from medicinal plants has progressed from the formulation of crude drugs to the isolation of active compounds in drug discovery. As practiced today, pharmacognosy involves the broad study of natural products from various sources including plants, bacteria, fungi, and marine organisms. Pharmacognosy includes both the study of botanical dietary supplements, including herbal remedies (Tyler, 1999 and Cardellina, 2002), as well as the search for single compound drug leads that may proceed through further development before considered as approved medicines. Drug discovery from medicinal plants is most frequently associated with the second of these two endeavors.

Pharmacognosy is closely related to botany and plant chemistry and indeed, both originated from the earlier scientific studies of medicinal plants. As the late as the beginning of the 20th century, the subject had developed mainly in the botanical side, being concerned with the description and identification of drugs with their history, commerce, collection, preparation, and storage. Such branches of pharmacognosy are still of fundamental importance, particularly for pharmacopoeial identification and quality control purposes, but rapid development in other areas has enormously expanded the subject (Trease and Evans, 2005).

Large numbers of plants are constantly being exposed for their pharmacognostic value as the plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. Precise identification of botanicals for use in herbal medicines is both a fundamental and regulatory requirement. Botanical identification is the primary means by which a plant is accurately identified and must be done by someone with the requisite skills, ideally a formally trained botanist. From earliest times until the mid-19th
century much attention was given to plant identification, specifically so that adulterations could be avoided. The very early works suffered botanically because of the relative infancy of botany as a science, but the later works were drawn with exquisite detail, providing medical professionals with a relatively high degree of accuracy to ensure the identity of their materia medics (Mukherjee and Houghton, 2009b). The increasing demand for herbal medicines both in the developing and developed countries inevitably led to maintaining the quality and purity of the herbal raw materials and finished products. The standardization problem relating to the herbal drugs arises from the complex composition of drugs that are used in the form of whole plant, plant parts or extracts obtained there from. To ensure reproducible quality of an herbal remedy, proper control of the starting material is utmost essential. Usually plants cannot be identified to species using only rhizomes, roots or barks, which for many medicinal plants are the parts found in market. As a result, the development of evermore sophisticated or molecular methods to be employed in quality control has become necessary, especially in such morphologically problematic species.

Such trials require complete characterization of the raw materials and finished product of the herbal drug being investigated to ensure its identity, purity, quality, consistency, and reproducibility. Without such knowledge, the findings of any clinical trial will be subject to question as well as be irreproducible. Testing methodologies that are applied to medicinal plants include botanical, macroscopic, microscopic, chemical and molecular methods of testing, which provide analytical tools that can be used for full botanical product characterization.

The pharmacognostic study of Anogeissus latifolia under investigation constitutes an important feature of organoleptic evaluation. The leaves are alternate or subopposite, elliptic or oblong-elliptic, obtuse or very often shortly cuspidate, glabrous when fully grown, pale dull glaucous-green, base usually rounded, midrib prominent. Flowers sessile, in small dense heads; peduncle one or
more from the same axil, branched, not much longer than the petioles. It is an erect tree reaching 18-21m; bark smooth, light colored; young parts glabrous or silky pubescent. Seeds are solitary.

Transverse section of the *Anogeissus latifolia* leaf shows a typical dicotyledonous leaf structure having upper and lower epidermis with cuticle, the upper epidermis is multilayered showing the xerophytic character. The cells are thin walled but lower epidermis discontinuous due to the presence of stomata. In between the two epidermal layers mesophyll is present, differentiated into palisade parenchyma and spongy parenchyma. The microscopy of stem shows secondary growth in which outermost layer form the periderma. Just below the periderma many layered zone *viz.* Secondary cortex and phloem are seen. Vascular bundles are conjoint collateral and open. After secondary growth all the vascular bundles are joined together with the help of cambium. So it looks like a compact structure showing metaxylem, protoxylem with prominent secondary medullary rays. Parenchymatous pith is very small in the centre.

Leaf surface data for *Anogeissus latifolia* were 07.70 ± 0.36, 12.53 ± 0.24, 05.66 ± 0.11, 05.30 ± 0.48 and 03.58 ± 0.65 for stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number respectively. Powder of *Anogeissus latifolia* leaf and stem showed extractive values of 4.76, 0.98, 10.9, 16.91 and 0.79, 0.2, 9.33, 8.78% for petroleum ether, chloroform, ethanol and aqueous extracts respectively. The total ash value, acid insoluble ash value and water-soluble ash value were 11, 2.85, 4.5 and 10.9, 0.65 and 0.9 % for leaf and stem respectively.

Pharmacognostic study involving morphological and organoleptic identification is the oldest, simplest and cheapest of all methods, thus to be preferred when its use is feasible along with the other parameters like ash value, extractive value and qualitative chemical tests serve as source of information.
Hence, the studies on pharmacognostic parameters are useful tools to determine the purity of plants and to avoid adulteration in the process of commercialization of raw material. The numerical values, stomatal number, stomatal index, palisade ratio, vein islet and vein termination numbers (Wallis, 1965) which are used for identification purposes are particularly useful in determining the purity and botanical sources of certain drugs of vegetable origin because these values are based upon the microscopic structure of leaves, they are fairly constant for the leaf of any particular plant and are used as reliable character by which the botanical origin of a leaf can be established, especially when concerned with closely related species.

The determination of the amount of any organic and inorganic constituents which may be present in any plant to which its value or therapeutic activity is attributed to proximate values like extractive and ash values (Remington, 1980). It is a good indicator of previous extraction of water-soluble salts in the drug or in correct preparation.

In view of establishing the identity and characterizing the plants for their purity, almost all the medicinal plants have been subjected to pharmacognostic evaluation and such measures are indespensible for any prospective pharmacological screening, further drug discovery and development. Hence there are umpteen number of investigations under taken utilizing several medicinal plants viz. Coleus forskohlii (Shrivastava et al. 2002); Actaea racemosa L. (Applequist, 2003), Uncaria tomentosa and Uncaria guianensis (Gattuso et al. 2004), Maytenus ilicifolia, (Duarte and Debur, 2005), Gisekia pharmacioides (Musa et al. 2006), Crateva nurvala (Sikarwar, 2009), Annona squamosa Linn. (Sharma et al. 2009), Holoptelea integrifolia (Padmaja, 2009), Polygonum nepalense (Rakesh and Garg et al. 2011), Ficus hispida (Ravichandra et al. 2011) and Xanthium strumarium (Bhogaonkar et al. 2012).
2. PHYTOCHEMISTRY

The use of plants for treating various diseases is an age-old practice in a large part of the world especially in developing countries where there is dependence on traditional medicine for a variety of diseases. Long before the advent of modern medicine, herbs were the mainstream remedies for nearly all ailments. Microorganisms and medicinal plants are rich sources of secondary metabolites, which are sources of useful drug and other bioactive products (Mark, 2004). Interest in plants with antimicrobial and other therapeutic properties has revived as a result of current problems such as resistance and toxic side effects, associated with the use of the antibiotics and other synthetic drugs. The primary benefits of plant-derived medicines are that they are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment (Iwu et al. 1999). Usually these natural products are extracted and isolated from plants. These extracts show various medicinal properties virtually for every ailment of humans. Furthermore, the discovery and the isolation of leading structures of the active plant metabolites have provided a unique starting point for chemical modification in an attempt to improve their activity, pharmaco kinetics and human safety (Kaur et al. 2006).

Natural products have played an important role as new chemical entities (NCEs) approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived. Another 20% of NCEs during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products (Newman et al. 2003). Combining these categories, research on natural products accounts for approximately 48% of the NCEs reported from 1981–2002. Natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereocenters that can be challenging synthetically (Clardy and Walsh, 2004; Nicolaou and Snyder, 2004; Peterson and Overman, 2004 and Koehn and Carter,
Many structural features common to natural products (e.g. chiral centers, aromatic rings, complex ring systems, degree of molecule saturation, and number and ratio of hetero atoms) have been shown to be highly relevant to drug discovery efforts (Lee and Schneider, 2001; Feher and Schmidt, 2003; Clardy and Walsh, 2004; Piggott and Karuso, 2004 and Koehn and Carter, 2005). Furthermore, drugs derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists.

The criteria used for selecting plant for investigation were based on: 1) traditional medicinal information (ethno-pharmacological knowledge); 2) chemical composition of the plant species; and 3) literature reports on plant extract's pharmacology and ethno medical claims.

In the present investigation, the preliminary phytochemical analysis of the plant extract revealed the presence of several bioactive compounds viz. sterols, alkaloids, tannins, flavonoids, cardiac glycosides and triterpenes in A. latifolia which therefore encourages further studies on these compounds for pharmacological properties.

In the phytochemical studies, the shade dried powdered leaf of the plant was subjected to Soxhlet extraction with different solvents viz. petroleum ether, chloroform, acetone, ethyl acetate and methanol. The methanol fraction was chromatographed over silica gel column, eluted with chloroform and with increasing concentrations of ethyl acetate in chloroform. The eluent from column chromatography was monitored by TLC, fractions with similar TLC patterns was combined to yield single spot PV4001. The latter was purified further by preparative TLC in 7:3 chloroform and ethyl acetate which resulted in a white colored compound and crystallized in a desiccator. The structure of the isolated
compound was determined by IR, Mass and $^1$H-NMR spectral analyses. The PV4001 was identified as Gallic acid.

The shade dried powdered bark of *Anogeissus latifolia* was extracted with methanol. The concentrated extract was dissolved in water and successively extracted with hexane, ethyl acetate and 1-BuOH. The butanol extract was evaporated under vacuum to yield flavonoidal fraction, which gave positive Shinoda test. The extract was subjected to purification over silica gel column using chloroform: acetone: formic acid as eluent; which yielded the compound PV4002. The structure of the isolated compound was determined by IR, Mass and $^1$H-NMR spectral analyses and identified as Myricetin.

Reddy *et al.* (1965) have isolated gallic acid from leaf acetone extract and myricetin from heartwood of *Anogeissus latifolia*. Other investigators also isolated gallic acid (Spiros Kambourakis *et al.* 2000 and Pawar and Surana, 2010) and myricetin (Hideaki matsuda *et al.* 2000, Lawrence *et al.* 2005 and Zeb Saddique *et al.* 2011). Phytochemical studies in the species previously resulted in isolation of tannin, (+) leucocyanidin and ellagic acid from the bark, sapwood and heart wood by Reddy *et al.* (1965), whereas, Deshapande *et al.* (1976) isolated 3,3'-di-O-methylellagic acid-4'-β-D-Xyloside and 3,4,3'-tri-O-methylflavellagic acid-4'-β-D-glucoside from stem bark. Steroid, β-sistosterol and a triterpenoid, 3-β- hydroxy-28-acetytaraxaren were isolated from the ethyl acetate fractions of stem bark of *A. latifolia* (Rahman *et al.* 2007).

Gallic acid is an organic acid found in a variety of foods and herbs that are well known as powerful antioxidants. Gallic acid reported to have anti-fungal and anti-viral properties. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as an astringent in cases of internal haemorrhage and used to treat albuminuria and diabetes (Tsao and Makepeasce, 1951 and Fiuza, 2004).
Myricetin is a naturally occurring flavonol, a flavonoid found in many fruits, vegetables, herbs, as well as other plants. Myricetin has antioxidant properties. *In vitro* research suggests that myricetin in high concentrations can modify LDL cholesterol such that uptake by white blood cells is increased. Studies also correlated high myricetin consumption with lowered rates of prostate cancer and reduced the risk of pancreatic cancer (Koo Hui Miean and Suhaila Mohamed, 2000, Knekt, 2002 and Nothlings, 2007).

3. PHARMACOLOGY

**Hepatoprotective Activity**

Several ancient systems all over the world have cited various herbs for the management of liver disorders and were used as hepatoprotective agents in the folklore medicine. However, the exact mechanism by which they protect the liver has not been studied extensively. Some studies conducted on hepatoprotective plants revealed that, the activity is because of inhibition of free radical-damage to the cells (Sultana et al. 2005). The involvement of free radicals such as superoxide anions and hydroxyl radicals and, other reactive oxygen species like hydrogen peroxide in various diseases has been established. The metabolism of certain pesticides, drugs, cigarette smoke and various other pollutants generate a number of reactive oxygen species and free radicals in the biological system. These radicals cause depletion of antioxidant enzymes and induce lipid peroxidation in the liver. Some studies show that the herbs may promote the antioxidant defense system to prevent CCl₄ induced hepatic damage (Koul and Kapil, 1999).

Elevation of serum markers are a known effect of CCl₄ toxicity and used as biochemical parameters of liver damage (Sturgill, 1997). The toxicity produced by CCl₄ is mediated through free radical mechanism. CCl₄ induced hepatic damage is due to its cytochrome P450 enzyme system catalysed hepatic conversion into highly reactive trichloromethyl radical, which upon reaction with oxygen radical
gives trichloromethyl peroxide radical. This radical forms covalent bond with sulfahydryl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl₄ (Cotran et al. 1994 and Kaplowitz et al. 1986), by encouraging the auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane, thus altering the permeability of the liver cell membranes and hepatic tissue destruction (Handa and Sharma, 1990). Hepatocellular necrosis leads to high level of serum markers in the blood. From the results of the present investigation it was evident that the sequential extracts of Anogeissus latifolia were able to reduce the elevated biochemical parameters due to the hepatotoxin administration. The reduced levels of total proteins in CCl₄ induced hepatotoxicity is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Recknagel, 1967). Reduction in the levels of AST, ALT and ALP towards the normal value is an indication of regeneration process. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al. 1987). Reduction of raised bilirubin level suggests the stability of the biliary function during injury with CCl₄. Estimations of serum bilirubin is the most sensitive test because it confirms the intensity of the hepatic damage determinations in serum and is used for the diagnosis, differentiation and follow up of jaundice. The protein levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis (Elliot and Strunin, 1993). Furthermore, serum proteins are affected both quantitatively and qualitatively in liver diseases and such changing levels of serum protein thus provide valuable indices of severity, progress, and prognosis in hepatic disease.
(Kagan, 1943 and Henry, 1986). The protective effect exhibited by the extracts was comparable to standard drug silymarin.

The histopathological observations in CCl₄ rats showed severe necrosis, with disappearance of nuclei. This could be due to the formation of highly reactive radicals because of oxidative stress caused by trichloromethyl radicals. All these changes were very much reduced histopathologically in rats treated with different extracts but the effect is more prominent in case of methanolic extracts of leaf and bark of the plant. Thus, administration of methanol extracts revealed hepatoprotective activity of Anogeissus latifolia against the toxic effect of CCl₄, which was also supported by histological studies.

The present study reveals that the methanolic leaf and bark extracts possess significant hepatoprotective activity which may be attributed to the individual or combined effects of active constituents present in it. Several phytoconstituents viz. triterpenes, alkaloids, flavonoids, tannins, glycosides, etc. have been found effective in the hepatoprotection against CCl₄ induced hepatic toxicity (Baek, 1996; Tran, 2001; Vijyan, 2003; Chin, 2004 and Madani et al. 2008). Moreover the presence of powerful antioxidant phytochemicals in the plant viz, gallic acid, myricetin etc. could be contributory to the hepatoprotection. Many investigators have evaluated the hepatoprotective effect of the phytoconstituents by comparing with standard drug silymarin (Formica and Regelson, 1995; Pin-Der-Duh, 1998; Chattopadhyay, 2003; Suja et al. 2005; Sureshkumar and Mishra, 2007; Avijeet et al. 2008; Manjunatha et al. 2008; Gamal et al. 2009; Ganga Rao and Jaya Raju, 2010; Sumitha and Thirunallasundari et al. 2011 and Gnanasekaran et al. 2012).

**Antihyperglycemic activity**

*Diabetes mellitus* is a complex, chronic disease characterized by an elevation of the level of glucose in the blood and is a global disease with a huge observes impact on health and mortality (Kannel and McGee, 1979). It occurs at
any time of the life from infancy to old age. It is a metabolic disorder characterized by disturbances in carbohydrates, protein and lipid metabolism and manifests complications like retinopathy, microangiopathy and neuropathy (Akhtar and Ali, 1980). The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. This number is likely to more than double by 2030. In 2005, an estimated 1.1 million people died from diabetes. Almost 80% of diabetes deaths occur in low and middle-income countries. Almost half of diabetes deaths occur in people under the age of 70 years; 55% of diabetes deaths are in women. Conventionally, insulin-dependent diabetes is treated with exogenous administration of insulin (Felig et al. 1995) and non insulin-dependent diabetes mellitus with synthetic oral hypoglycemic agents like sulphonylureas and biguanides (Rosac et al. 2002). However the hormone fails as a curative agent for complications of diabetes (Mukherjee et al. 1966) and synthetic oral drugs produce adverse health effects (Raheja, 1977). Different medicinal systems are using the active plant constituents as natural hypoglycemic medicine, came from the virtue of traditional knowledge. Herbal drugs are considered less toxic, relatively cheap and popular (Momin, 1987). Even today great opportunities are still open for scientific investigations of herbal medicines for cure of diabetes and its complications. In response to WHO’s (1980) recommendation for research on the beneficial uses of medicinal and crop plants in the treatment of diabetes mellitus, several investigations have been carried out which resulted in identifying many plants exhibiting positive activity (Shirwaikar et al. 2004).

In the present study, the results revealed that the leaf and bark extracts particularly methanol extracts of the plant *Anogeissus latifolia* possess potent antidiabetic activity by exhibiting effectiveness for blood glucose level reduction studied in STZ deoxy-5{[(methyl-nitrosoamino) carbonyl]-amino}-D glucopyranose induced diabetes. STZ produces a selective toxic effect on β-cells
of pancreas and induces diabetes in most laboratory animals (Lawn et al. 1979 and Oliver and Oval, 1980). It showed that oral administration of methanolic extracts of the plant produced remarkable decrease in blood glucose on 5th and 7th hour and 7th day. This reduction of blood sugar may be because of the extract either protected the cell from the toxic effect of STZ or the cells recovered after the initial injury (Paramesha et al. 2009a and Parvathi et al. 2009a). The phytochemical analysis of the extracts of the plant showed the presence of constituents viz. triterpenes, alkaloids, flavonoids, tannins, flavones, glycosides. The antidiabetic activity may be due to the presence of active principles in the plant, containing flavonoids and steroids. Further, flavonoids are also been reported to be effective in few diabetic complications such as heart disease, neuropathy and retinopathy (Ray et al. 2006). Gallic acid, one of the isolated phytochemical from the plant has been reported to possess antidiabetic properties (Huang Tom et al. 2005 and Punitahavathi et al. 2011). Similar observation on antidiabetic efficacy was reported by several workers using plant extracts of different plants viz. Chakravarthy et al. (1980); Ray et al. (2006); Paramesha et al. (2009a); Parvathi et al. (2009a) and Prerona Saha et al. (2011) and Sanjay Kumar Karan et al. (2012).

**Hypolipidemic activity**

Hyperlipidemia is characterized by elevated serum total cholesterol, low density and very low-density lipoprotein cholesterol and decreased high-density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (Saravanan et al. 2003). Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (Grundy, 1986). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Smith et al. 1993). Among these, hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (Kaesancini
et al. 1994). Currently available hypolipidemic drugs have been associated with a number of side effects (Brown, 1996). The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Speight and Averyis. 1987). The quest for finding the new safe and effective drug for dyslipidaemia is going to be a continuous process amongst the scientific fraternity. Herbs have been used as food and for medicinal purposes for centuries. Contemporary research has focused on various herbs that possess hypolipidaemic properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease. Apart from the synthetic modern drugs, there are efforts to find out herbal drugs possessing lipid lowering activities (Berliner and Heinecke, 1996).

Gum ghatti an exudate from Anogeissus latifolia is a complex polysaccharide of high molecular weight. It occurs in nature as a mixed calcium, magnesium, potassium and sodium salt. Complete hydrolysis has shown that it is composed of L-arabinose, D-galactose, D-mannose, D-xylene and D-glucoronionic acid in a molar ratio of 10:6:2:1:2 plus traces less than 1% of 6-deoxyhexose (Fahrenbach et al. 1996). The results of the present experiment revealed that feeding of atherogenic diet increased the serum total cholesterol, triglyceride and decreased serum HDL-cholesterol level when compared to normal group. Administration of 250mg/kg of gum ghatti has shown significant (p<0.01) decrease in only serum triglyceride level whereas in case of 500 mg/kg significant decrease has occurred both in the level of total cholesterol and triglyceride. In case of 750mg/kg, significant decrease has occurred both in the level of total cholesterol, triglyceride and significant elevation in the HDL level. Thus compared to all the three concentrations 750mg/kg has shown efficient and comprehensive lipid lowering ability.

Atherogenic diet induced hyperlipidemic model has been successfully employed for the evaluation of hypocholesterolemic effect of protein (Salil and
Rajmohan, 2001). Ahluwalia and Amma (1998) found that feeding of oleoresin of gum guggal (Commiphora mukul) lowered the total cholesterol and its fractions in lipoproteins. Sewaga Kotaro et al. (1998) have reported cholesterol lowering effects of Psyllium seed associated with urea metabolism. Hypolipidemic effect of the proteins, gums, saponins and bita sitosterol have been reported by several authors. Faulty diet is a very common cause of heart disease. Particularly, with an increase in inclination towards fast foods which are rich in saturated fats, an increase in coronary heart disorder (CHD) is being observed in the developing countries since past few decades (Kulkarni and Kaur Gurpreet, 1999). The administration of dietary fibre increased the amount of fecal bile acid with cholesterol reduction in the blood (Marlett et al. 1994). It was also demonstrated that the fermentation products of dietary fibre, short chain fatty acids, led to reduction of hepatic cholesterol synthesis (Bridges et al. 1992 and Kishimoto et al. 1995). In the present experiment high molecular weight complex polysaccharide nature of gum ghatti could be attributed to its lipid lowering activity. The results of this study warrants further investigation on the exact mechanism of action of hypolipidemic activity of gum ghatti of Anogeissus latifolia.

Several investigators have explored the lipid lowering abilities of many medicinal plants viz. Curcuma longa (Godkar et al. 1996); Psyllium seed (Kotaro Segawa et al. 1997); Fenugreek (Prasanna et al. 2000); Allium sativum (Augusti et al. 2001); Ginkgo biloba (Arun Kumar Dubey et al. 2004); Clemeo felina (Nagarajan et al. 2005); Salacia oblonga and Salacia reticulata (Rabbani et al. 2006); Camellia sinesis (Saravana Kumar et al. 2007); Aloe vera (Mamata chandhrakar et al. 2008); Carica papaya (Adeneye and Olagunju, 2009); Mucuna purpuriens (Murugan and Uma Maheshwara, 2009); Mimosa pudica (Rekha Rajendran and Ekambaram Krishnakumar, 2010); Asparagus racemosus (Ramachandran Vadivelan et al. 2011); Vebesina encelioides (Rakesh Sindhu
et al. 2011); Pleurotus ostreatus (Nuhu Alam et al. 2011) and Nyctanthes arbortristis (Krishna Murti et al. 2012).

Anti-inflammatory activity

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbial agents and is the body's effort to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair (Mary, 1997). Upon interaction of foreign pathogens with innate immune cells like macrophage or monocytes, inflammatory immune response is trigger off. A series of pro-inflammatory mediators, specialized cytokines, prostaglandins, chemokines are produced as a result in a way to amplify the inflammatory response (Beg, 2002).

Administration of carrageenan to the control group of rats showed rise in paw volume where standard drug Indomethacin treated group showed a significant reduction (p<0.01) in paw edema volume at different time intervals viz. 1, 2 and 3h. Oral administration of sequential extracts belonging to leaf and bark of Anogeissus latifolia has shown reduction in inflammation induced by carrageenan. Chloroform leaf extracts exhibited significant reduction (p<0.01) whereas, the reduction in paw volume was more prominent for methanol leaf extract by recording lower mean values and percent inhibition at all the time intervals under study. Similarly bark extracts also exhibited a significant (p<0.01) anti-inflammatory activity by reducing the paw volume and percentage inhibition for chloroform and methanol extracts. The latter manifested potent anti-inflammatory effect when compared to chloroform extract. It is evident from the data that the reduction in inflammation is positively correlated with duration wherein highest reduction in paw volume was observed at 3h. Further, perusal of results revealed clear cut superiority of bark over leaf for anti-inflammatory activity in terms of both mean values as well as inhibition percentage.
The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Seibert, 1994). The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Vinegar, 1969). The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also to histamine and serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The second phase is sustained by prostaglandins released and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages component (Crunkhorn and Meacock, 1971; Brito and Antonio, 1988) and play a major role in the development of the second phase of inflammatory reaction. In the present investigation, methanolic and chloroform extracts of leaf and bark showed remarkable effect at 1, 2 and 3h. In general, the anti-inflammatory activity is more evident at the later phases of time interval. Therefore, it can be inferred that the inhibitory effect of extracts on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. The phytochemical screening revealed the presence of flavonoids in chloroform bark and methañolic extracts of leaf and bark of the plant is known to inhibit prostaglandin synthetase (Ramaswamy, 1985). Further, Myricetin glucuronide (MGL) a form of Myricetin has been reported to exert a marked and dose-dependent anti-inflammatory effect in acute and chronic models of inflammation induced by carrageenan (Hiermann et al. 1998). Similar investigations on plants as anti-inflammatory agents has been carried out by various workers viz. Curcuma amada (Mujumdar et al. 2000); Goniothalamus andersonii (Shigeo et al. 2001); Clitoria fairchildiana (Pereira da Silva and Paz Parente, 2002); Calendula officinalis, Hypericum perforatum, Plantago lanceolata and Glycyrrhiza glabra (Herold et al. 2003); Alchornea cordifolia (Mavar et al.
2004); *Vitex negundo* (Rasadah et al. 2005) *Bacopa monniera* (Shabana, 2006); *Ruta graveolens* (Ratheesh and Helen, 2007); *Putranjiva roxburghii* (Wantana, 2009); *Magnolia ovate* (Candida, 2009) and *Boswellia Serrata* (Ramakrishnan et al. 2011) and *Leptadenia pyrotechnica, Haloxylon salicornicum* and *Ochradenus baccatus* (Saleh Ibrahim Alqasoumi et al. 2012).

**Analgesic activity**

Pain, even though is an unpleasant sensation, is mainly a protective mechanism for the body (Kanodia and Das, 2008). It is a consequence of complex neurochemical processes in the central and peripheral nervous systems. Typically, it is a direct response to an event associated with tissue damage, such as injury, inflammation or cancer or it can also occur as a consequence of brain or nerve injury. Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively.

The nonopioid analgesics relieve pain without interacting with opioid receptors, reduce elevated body temperature, possess anti-inflammatory property and are non-addicting drugs. These effects are achieved with doses that do not produce significant depression of CNS. The NSAIDs can be classified mainly into two groups, namely, non-selective COX inhibitors (acetyl salicylic acid, paracetamol, phenylbutazone, diclofenac, ibuprofen, piroxicam etc.) and selective COX-2 inhibitors (nimesulide, meloxicam, celecoxib, rofecoxib). Though these drugs have different chemical structures, they produce qualitatively similar actions. During inflammation, pain and fever, arachidonic acid is liberated from phospholipid fraction of the cell membrane. This acid is then converted via cyclooxygenase (COX-1 and COX-2) pathways to prostaglandins. These prostaglandins sensitize blood vessels to the effects of inflammatory mediators that increase permeability. The prostaglandins particularly PGE and PGI produce hyperanalgesia associated with inflammation. They sensitize the chemical receptors of the afferent pain endings to other mediators such as bradykinin and
histamine. Further, release of prostaglandins in the CNS may lower the threshold of the central pain circuits.

Opioid are drugs which have morphine like action viz. relief of pain and depression of the CNS. The opioid drugs produce their effects by combining with opioid receptors, which are widely distributed in the CNS and other tissues. The opioid receptors have been classified into mu, delta, kappa (K₁ and K₂) and sigma types. The vast majority of opioid drugs used as analgesics are agonists at mu receptors. The major drawbacks of these opioid analgesics are the development of tolerance and physical as well as psychological dependence.

Analgesic effect of sequential extracts of the plant viz. *Anogeissus latifolia* on tail flicking in rats at different time intervals viz. 0, 30, 60, 90 and 120 min reveals that rats treated with petroleum ether and chloroform of leaf recorded significant (p<0.01) increase in reaction time over the control at 90 and 120 min only, while leaf methanol extract at 30, 60, 90 and 120 min. Among bark extracts, petroleum ether and chloroform extracts elevated the reaction time significantly (p<0.01) at 60, 90 and 120 min time intervals, whereas methanol bark extract exhibited similar effects at 30, 60, 90 and 120min indicating potent analgesic effect. It was noted that the activities of various extracts are more effective in later phases of treatment and activity was shown in the order of petroleum ether leaf< petroleum ether bark< chloroform leaf<chloroform bark<methanol leaf< methanol bark. Further, the bark extracts of corresponding solvents proved to be effective in terms of analgesic property compared to leaf extracts.

The phytochemical screening of the extracts has revealed the presence of triterpenoids, steroids tannins, alkaloids and flavonoids. The variable analgesic
activity in different extracts could be due to the presence of phytochemicals. Analgesic effects of flavonoids steroids and tannins have been well documented (Das, 1989). The presence of Myricetin, which is one of the isolated constituent in the present research programme could be contributory to the analgesic activity as it was reported to be a potent COX-1 inhibitor with anti-platelet activity (Shu-Jun Wang et al. 2010). The present work corroborates investigations carried out in several medicinal plants viz. *Sida acuta; Stylosanthes fruticosa, Toona ciliate, Bougainvilla spectabilis, Ficus glomerata* and *Polyalthia longifolia* (Malairajan, 2006); *Mahonia owakensis* (Jung Chao, 2009); *Argyreia speciosa* (Bachhav, 2009); *Citrus decumana* (Shailja et al. 2009); *Kaempferia galanga* (Amberkar Mohanbabu et al. 2011) for analgesic activities.

**Anthelmintic activity**

Helminthiasis infections are prevalent in people all over the world, but most common in the tropical and subtropical regions. The World Health Assembly, in a number of resolutions has emphasized the need to the use of natural products with therapeutically proven efficacy particularly in patients residing in tribal areas who are very much prone to the attack of several infections due to lack of knowledge about proper sanitation (Ghosh et al. 2006). Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects. For example, plants that contain condensed tannins, a class of phenolic secondary metabolites, have these effects (Jalalpure et al. 2003). Anthelmintics are those drugs that are used in expelling out the worms that are parasitic in nature by either stunning them or by killing them. They are also known as vermifuges or
vermicides. Search for anthelmintic factor in plants therefore remains a potential area of investigations.

The anthelmintic activity was evaluated on Indian adult earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being (Vidyarthi, 1977, Thorn *et al.* 1977 and Vigar, 1984). Most anthelmintics target the neuro-musculature and therefore similarities here become of particular importance. The wiring diagram for the neuromuscular system is similar between *Pheretima posthuma* and the parasitic nematode *Ascaris suum* (Angstadt *et al.* 1989).

The present study revealed that the sequential extracts of leaf and bark of *Anogeissus latifolia* on Indian earthworm *Pheretima posthuma* at different concentration *viz.* 10, 20, 30, 40 and 50 mg/ml possess potent anthelminthic property in a dose dependent manner for the parameters studied *viz.* paralysis and death which is quite comparable with standard anthelmintic drug in the organism. Among the extracts of leaf, pet ether has shown the maximum reduction in time for paralysis, followed by chloroform and methanol while among bark, chloroform has shown maximum reduction for the same parameter. Similarly, in regard to the time taken to death (minutes), among the extracts, pet ether has shown the maximum reduction in time followed by chloroform and methanol in leaf while, the effect was maximum in chloroform followed by pet ether and methanol. The result indicates a negative correlation between time and concentration of the extracts. From the data it is evident that among the two sources of extracts bark extracts proved to be more effective when compared to leaf extracts in terms of anthelmintic activity with respect to solvents used.
The major effect of anthelmintic compounds could be due to decrease in motility, paralytic action, damage to the mucopolysaccharide membrane and on the neuromusculature of helminthes worms. The metabolic pathways in general and carbohydrate pathways in particular and neuromuscular co-ordination are the major targets (Dhar, 1965). The effect is due to the presence of active principles in the plant extracts (Ghosh et al. 2007). This act as potent anthelmintic, because the extracts of the plant contains flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins. Specifically, tannins present in the extracts could be attributed to profound anthelmintic activity (Athnasiadou et al. 2001; Shivkar and Kumar 2003; Mali et al. 2007; Khadatkar et al. 2008; Paramesha et al. 2009b; Parvathi et al. 2009b; Asha et al. 2009; Deore and Khadabade, 2010 and Muhammad Erfan Uddin et al. 2012). Tannins are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death (Thompson, 1995). The anthelmintic activity of the various extracts of the plant may be attributed to similar reasons.