I. GENERAL INTRODUCTION

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. The development of these traditional systems of medicines with the attribute to safety, efficacy, and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare (Mukherje et al., 2006). Such drugs are very prone to adulteration if the supply of crude drug is poor. This adulteration could be prevented by various pharmacognostic parameters. Pharmacognostic study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostic evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs.

Phytochemistry or plant chemistry is a distinct discipline somewhere here in between natural product organic chemistry and plant biochemistry and is closely related to both. Plants are potent biochemists and have been components of phytomedicine since from ancient time. Plant based bioactive constituents can be derived from any part of the plant like bark, leaves, flowers, roots, rhizome, fruits, seeds, etc i.e. any part of the plant may contain active components. The methodical screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh et al., 2006). In India, Malaysia and Thailand, about 150 wild plants species have been identified as sources of emergency food (Nesamvuni et al., 2001). Most of the rural people dependent on the surrounding forests for their day-to-day needs. Proximate and nutrient analysis of wild edible plants plays a crucial role in assessing their nutritional significance (Pandey et al., 2006).
Among leading health problems, infectious diseases account for 41% of the global disease burden along with noninfectious diseases (43%) and injuries (16%) (Noumedem et al., 2013). Mastitis is an inflammation of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits et al., 2000; Sharma et al., 2012). Infection of the cow’s udder (bovine mastitis) has remained one of the major constraints to growth of the dairy industry in India. Amongst cattle diseases, bovine mastitis is a serious problem, which affects the basic income of the farmers depleting their dairy sources (Al Qumber et al., 2006; Mubarack et al., 2011). Financial losses due to Mastitis occur in both subclinical and clinical stages of the disease and leads to spoilage of milk and milk yield. Tuberculosis (TB) is a deadly infectious disease caused by Mycobacterium tuberculosis. There were an estimated 8.8 million incident cases of TB (8.5 - 9.2 million) globally in 2010. Most of the estimated number of cases in 2010 occurred in Asia (59%) and Africa (26%). India alone accounted for an estimated one quarter (26%) of all TB cases worldwide (WHO, 2011). Tuberculosis is the major opportunistic infection of HIV/AIDS in developing countries (Joseph et al., 2006). With an increase in the number of people living with HIV and AIDS in India, the incidence of HIV/TB coinfection is expected to be on the rise. The high incidence of multidrug-resistant TB (MDRTB) in India is yet another issue that poses a challenge to infection control measures (Zignol et al., 2006; Sharma et al., 2011). Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Shiota et al., 2004; Abu-Shanab, 2006).

In a developing country like India, where disease management is big issue the knowledge of plant derived antioxidant could reduce the cost of health care. Antioxidants were used to protect human beings from the ill effects of oxidative stress.
that is exerted by enhanced production of pollutants. The body has several mechanisms to counteract oxidative stress by producing antioxidants naturally regenerated either in situ or externally supplied through the foods (Halliwell et al., 2007).

Free radicals or reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite can damage the body by cellular or oxidative stress. This leads to the development of diseases like diabetes, cancer and cardiovascular (Valko et al., 2007). Free radicals generated in the body can be removed by its own natural antioxidant defense systems that include superoxide dismutase, glutathione peroxidase, and catalase. However, endogenous antioxidant defenses are not completely efficient. Therefore, dietary antioxidants are required to lessen the overall effect antioxidants stress due to excessive free radicals occurring in our system (Rajkumar et al., 2011).

Interestingly, the natural antioxidant compounds tend to be safer and have less or no side effects. In addition, they also possess anticancer antidiabetic and antiviral properties (Lim et al., 2007). Significant antioxidant properties have been recorded with phytochemicals that are necessary for the curing of diseases like cancer and diabetes (Abdel et al., 2009). The efficacy of plant extract as an antioxidant is long been well established and many more plants or plants extracts are under way. A plant contains a rich source of free radical scavenging molecules such as phenols, flavonoids, terpenoids that hold promising antioxidant properties (Upadhyay et al., 2010). These results clearly showed that plant derived compounds might have significant protective effects in vivo (Rajkapoor et al., 2010). Therefore, the
evaluation of antioxidants activity of various plants extracts is considered as an important step in the identification of their ability to scavenge the free radicals.

Diabetes is a metabolic syndrome characterized by increase in blood glucose level. The impaired action or absolute deficiency of insulin results in imbalance of glucose metabolism and leads to diabetes mellitus. Oxidative stress, through the production of reactive oxygen species (ROS), reported as the root cause for the development of insulin resistance, beta-cell dysfunction, impaired glucose tolerance (Wright et al., 2006). These synthetic drug molecules cause some side effects (flatulence, diarrhea and hepatitis). Recently, it has been shown that phenolic compound play a potential role in management of diabetes (Nampoothiri et al., 2010). Hence, the present investigation was undertaken to study the pharmacognostic analysis, antimicrobial, antituberculosis, antioxidant, anticancer and antidiabetic activity of rhizome of Curcuma pseudomontana J.Graham.
II. REVIEW OF LITERATURE

Time immemorial plants were used in the treatment of diseases and for invigorating body systems in Indian. Plants have a vast potential for their use as curative medicine. In India, medicinal plants are widely used by the people both directly as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda and Unani and indirectly in the pharmaceutical preparations (Srinivasan et al., 2001). India has about 4.5 million plant species and among them, thousands of plants were reported to have medicinal properties against human diseases. Even though traditional medicinal healers have used medicinal plants for treatment of ailments for hundreds of years, there has always been a remaining question in scientific community about their therapeutic usefulness. As a result, the biological activities of many medicinal plants have been examined, even though the vast majority of medicinal plants remain to be examined for their bioactive components and pharmacological effects.

Pharmacognostic analysis

The word “pharmacognosy” was coined in the early 19th century to designate the discipline related to the study of medicinal plants (Ganzinger, 1982). The science of pharmacognosy became aligned with botany and plant chemistry, and until the early 20th century, dealt mostly with physical description and identification of whole and powdered plant drugs including their history, commerce, collection, preparation, and storage. The use of herbal medicines continues to expand rapidly across the world. According to WHO, 80% if the rural population in developing countries depend on traditional medicines to meet their primary health care needs (Bannerman et al., 1993). Advances in organic chemistry added a new dimension to the description
and quality control of these drugs, and the discipline has since expanded to include discovery of novel chemical therapeutic agents from the natural world (Betz et al., 2011). The standardization of a crude drug is integral part of establishing its correct identity. Previous to any crude drug is included in herbal pharmacopoeia, pharmacognostic as well as other standard parameters must be established (Abere et al., 2007). Therapeutic efficacies of medicinal plants depend upon the quality and quantity of chemical constituents. It has been established that chemical constituents of a plant species vary with regard to climate and seasons (Bapodara et al., 2011). A number of different bases are used for morphological studies and a natural variation in these characteristics play an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as for as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs (Mukherjee, 2002).

Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine (Ahmad et al., 2009). In traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder is a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. (Kamboj, 2000). It is very important that a system of standardization is established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. World Health Organization (WHO) encourages, recommends and promotes traditional /herbal remedies in national health care programmers’ because these drugs are easily
available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards (Raina, 2003). Some of the standardization tests for herbal medicines are listed below (Ritch, 2000).

**Macro and microscopic examination:** For identification of right variety and search of adulterants.

**Foreign organic matter:** Remove of matter other than source plant to get the drug in pure form.

**Ash values:** It is criteria to judge the identity and purity of crude drug – Total ash, sulfated ash, water soluble ash and acid insoluble ash etc.

**Moisture content:** To check moisture content is helps in prevent degradation of product.

**Extractive values:** These are indicating the approximate measure of chemical constituents of crude drug.

**Crude fiber:** To determine excessive woody material criteria for judging purity.

Qualitative chemical evaluation: It covers identification and characterization of crude drug with respect to phytochemicals constituent.

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube et al., 2008). The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by
treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluidextracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation (Handa et al., 2008).

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh et al., 2006). Fresh or dried
plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. However, as many plants were used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40°C for 72 h. In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties (Das et al., 2010).

Plants generally produce many secondary metabolites, which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Fig.1). Secondary metabolites (compounds) have no apparent function in a plant’s primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against microorganisms, insects and higher predators and even other plants (allelochemics). In smaller quantities secondary metabolites were frequently accumulated in the plants than the primary metabolites (Karuppusamy, 2009; Sathishkumar and Paulsamy, 2009). In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary
metabolites (steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and the molecular weight are generally less than 2000.

**Plants with antimicrobial activity**

unknown, although it is thought that it could be because of the resurgence of TB due to HIV infection as well as Multiple Drug Resistant Tuberculosis (MDR-TB) due to inefficient management. Each year an estimated eight million new cases and two million deaths occur due to TB worldwide (Kishore et al., 2007). Tuberculosis is the major opportunistic infection of HIV/AIDS in developing countries (Joseph et al., 2006). Anti- Infection of the cow’s udder (bovine mastitis) has remained one of the major constraints in growth of dairy industry in India and abroad. Amongst cattle
diseases, bovine mastitis is a serious problem, which affects the basic income of the farmers depleting their dairy sources (Mubarack et al., 2011). Financial losses due to mastitis occur for both subclinical and clinical stages of the disease and leads to spoilage of milk and milk yield. Worldwide, Mastitis is associated with economic losses of 35 billion dollars annually. Clinical and sub clinical cases of mastitis usually treated with antibiotics intramammarily and parenterally. The continuous use of antibiotics for a long period may lead to multi drug resistance in causative organisms, which has resulted in the use of high doses of antibiotics and leads to the danger of increasing amounts of antibiotics residues in milk, a potential hazard (Gopinath et al., 2011).

Medicinal plants have been used for ages in developing countries as alternative treatment to health problems. India has a diverse flora and a rich tradition in the use of medicinal plants for antimicrobial applications. Many plant extracts have been shown to exert biological activity in vitro and in vivo, justifying research on traditional medicine focused on the characterization of antimicrobial activity of these plants (Mubarack et al., 2011). The efficiency of some of these plants/herbs has been tested against a range of causative agents of mastitis. In-vitro study conducted by Sahle indicated that Persicaria sengalensis, Cyphostema adenocaule and Cummis ficifolius, have shown some degree of growth inhibitory effect. Marisa et al., (2009) has screened ten herbal preparations; namely, Artemisia absinthium, Cymbopogon nardus, Symphytum officinale, Baccharis dracunculifolia, Solanum asperolanatum, Salvia officinalis, Bauhinia forficata, Calendula officinalis, Chenopodium ambrosioides and Senna macranthera on major isolates of bovine mastitis and also Mubarack et al., (2011) have conducted in-vitro tests of Acacia nilotica and
Acyranthus aspera on Staphylococcus aureus isolate and observed encouraging results.

**Plant with antituberculosis activity**

Tuberculosis (TB) is principally a disease of poverty, with 95 per cent of cases and 98 per cent of deaths occurring in developing countries (Sharma et al., 2004). Tuberculosis (TB) is a bacterial infection caused mainly by *Mycobacterium tuberculosis* (MTB). The development of paleopathology and paleoepidemiology in infectious diseases has proven the very ancient origin of this disease (Godreuil et al., 2007). TB is the most common cause of death due to a single infectious agent worldwide in adults. In 1993, the World Health Organization (WHO) took an unprecedented step and declared TB to be a global emergency (Sharma et al., 2004). The exact cause of this is tuberculosis drugs are a two-edged sword. While they destroy pathogenic *M. tuberculosis* they also select for drug resistant bacteria against which those drugs are then ineffective. Global surveillance has shown that drug resistant tuberculosis is widespread and is now a threat to tuberculosis control programs in many countries (Johnson et al., 2006).

Natural products including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists (Patwardhan et al., 2004). Humans have selected natural products as crude materials with efficacy against various diseases over many generations of practical experience. Such experiential evaluation is different from the scientific evaluation of western medicines and is underestimated sometimes. However many effective
medicines, including as morphine, aspirin, atropine, ephedrine, reserpine and digitoxin were developed from natural products (Kurokawa et al., 2010). Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Hoareau et al., 1999). In Ayurveda tuberculosis is known as Rajayakshma, Yakshma, Shosha, Kshaya (Vasanthakumari et al., 2007). Ayurveda, literally meaning the "Science of life and longevity" in ancient Sanskrit, is the one of the oldest healing system of India based on lifestyle, diet and herbs (Samy et al., 2008).

**Plants with Antioxidant potentials**

Damage to proteins, lipids and DNA by reactive oxygen species (ROS) and reactive nitrogen species (RNS) can lead to a variety of chronic diseases such as cancer, cardiovascular, inflammatory and age-related neurodegenerative diseases (Richardson, 1993; Borek, 1997). ROS/RNS can damage cell membranes, disrupt enzymes, reduce immunity (Ahsan et al., 2003) and induce mutation (Loft and Poulsen, 1996). ROS/RNS are by-products of normal aerobic metabolism and occur during mitochondrial/microsomal electron transport chain, phagocytic activity or generated from oxidase enzymes and transition metal ions (Aruoma et al., 1989; Nohl et al., 2003). Other sources of ROS/RNS are environmental factors such as pollution, exposure to UV rays of sun, cigarette smoke and even some kinds of food (Schroder, 2004). Oxidative damage induced by these reactive species is usually counteracted by antioxidant defense mechanism (Bagchi, 1998). Recent studies have shown evidence
that plant-based diets, particularly those rich in fruits and vegetables provide a considerable amount of antioxidant phytochemicals, which offer protection against cellular damage (Dimitrios, 2006).

In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Plants are potential sources of natural antioxidants and produce various antioxidative compounds that have therapeutic potentials. Antioxidant-based drug formulations are used for the prevention and treatment of many complex diseases.

Chirangini et al., (2004), have reported that rhizome extracts of some members of the medicinal Zingiberales are widely used in dietary intake as well as in the traditional system of medicine. Curcumin, the chrome orange yellow coloring compound present in turmeric rhizomes, has long been known to possess antioxidant property. Chirangini evaluated crude methanol extracts of the rhizomes of 11 species, including C. caesia for their antioxidant properties using sulphur free radical reactivity with curcumin as a reference indicator, C. caesia gave good degree of radioprotection.

The alcohol–water extract of Ichnocarpus frutescens leaves possessed 1, 1-diphenyl-2-picrylhydrazyl radical and superoxide anion radical scavenging activity (Kumarappan et al., 2007). Siddhuraju and Becker (2007) have reported the antioxidant and free radical scavenging activities of processed cowpea seed extracts, wherein the DPPH radical and ABTS cation radical scavenging activities were correlated with the ferric reducing antioxidant capacity of the extracts. The scavenging effect of Andrographis paniculata was demonstrated against DPPH and ABTS showing its ability to convert unpaired electrons to paired ones (Tripathi and
Kamat, 2007). A crude aqueous extract of Chlorophytum borivilianum has been shown to scavenge DPPH free radicals and decrease TBARS, revealing that it is a promising anti-stress agent as well as a potential antioxidant (Kenjale et al., 2007).

The antioxidant activity of the aqueous extracts of the leaves of Bauhinia forficata and Cissus sicyoides were determined using several different assay systems, namely (ABTS) decolorization, superoxide anion radical (O$_2^-$) scavenging and myeloperoxidase activity (Khalil et al., 2008). The methanol extract of Annona squamosa and Sapium macrocarpum showed two times more DPPH scavenging activity than the commercial antioxidant butylated hydroxyl anisole (Ruiz et al., 2008). An aqueous extract from Choerospondias axillaries showed a potent scavenging effect on DPPH (Wang et al., 2008). Methanol extract of bark, fruits and leaves of Ficus microcarpa exhibited excellent ABTS scavenging activity (Ao et al., 2008). Desai et al., (2008) have reported the free radical scavenging potential of the aqueous extract of roots of Baliospermum montanum by DPPH and nitric oxide (NO) scavenging assay, which showed a high concentration-dependent free radical scavenging activity.

The aqueous, methanol and ethanol extracts of Melissa officinalis, Matricaria recutita and Cymbopogan citrates were found to possess DPPH scavenging activity (Pereira et al., 2009). The methanol extracts of leaves and flowers of Lippia alba exhibited very significant DPPH radical scavenging activity compared to the standard antioxidant ascorbic acid (Ara and Nur, 2009). The methanol extract of Manikara zapota showed strong activity on scavenging DPPH radical, which implicated an essential defence against the free radicals (Kaneria et al., 2009). The hot water extract of Perilla frutescens stalk showed moderate DPPH radical scavenging abilities than
the leaf and seed extracts (Chou et al., 2009). The essential oils of *Myrtus communis* contained compounds such as 1, 8-cineole and methyl eugenol that showed considerable DPPH scavenging activities (Dukic et al., 2010).

Mohit Mangla et al., (2010) have investigated the antioxidant activity of methanolic extract of rhizomes of *C. caesia* using DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. This suggests that methanolic *C. caesia* extract had moderate IC$_{50}$ value as compared to Butylated Hydroxytoluene. Krishnaraj et al., (2010) were determined phenol content and antioxidant activity of *C. caesia* in comparison with *Curcuma amada*. The methanolic rhizome extracts of *C. amada* and *C. caesia* possess phenol. The reducing power and superoxide, ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] and DPPH radical scavenging activities of *C. caesia* were higher than *C. amada*. These results supported that the non-conventional *C. caesia* could be an economically. Karmakar et al., (2011) studied the methanolic *C. caesia* extract rhizome for some *in vitro* antioxidant activity. Effect of methanol extract of *Curcuma caesia* rhizome on ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) were evaluated *in vitro* methods like DPPH radical, hydroxyl radical, superoxide anion, nitric oxide, hydrogen peroxide, peroxynitrite and hypochlorus acid. Lipid peroxidation, total phenolic content was also measured by standard assay method. The extract show significant antioxidant activity in dose dependent manner.

Angel et al., (2012) have investigated antioxidant and antibacterial activities of oleoresins isolated from nine *Curcuma* species. Oleoresins were extracted from rhizomes of nine starchy *Curcuma* species (*Curcuma aeruginosa*, *C. amada*, *Curcuma aromatica*, *Curcuma brog*, *C. caesia*, *Curcuma malabarica*, *Curcuma*
rakthakanta, Curcuma sylvatica and Curcuma zedoaria) using dichloromethane and evaluated for antioxidant and antibacterial activity. Oleoresins from all the species exhibited high DPPH radical scavenging activity and ferric reducing power, which had good correlation with phenolic content (Das et al., 2012).

**Plants with anticancer properties**

Majority of human cancers are induced by environmental factors existing in the milieu. It has been projected that more than two-third of human cancers could be averted by lifestyle modifications including dietary changes (Surh, 2003). Epidemiological studies have strongly indicated that certain daily-consumed dietary phytochemicals could have cancer preventive effects against several forms of human cancers (Block et al., 1992). Diet has been regarded as a potential source of chemopreventive agents because of its likely protection following long-term administration to humans. Indeed, a number of natural compounds with inhibitory effect on cancer formation have been identified from diet or source of diet.

Plant derived compounds, in particular have a special place in anti-cancer therapy, and some of the new chemotherapeutic agents currently available for use in a clinical setting include paclitaxel, vincristine, podophyllotoxin and camptothecin (Gerzon, 1980; Kinghorn, 1993). Due to lack of effective drugs, cancer is a fatal disease rating the top three cause of death. Many of the chemotherapeutic agents sold for the treatment of cancer are highly expensive, mutagenic, carcinogenic and teratogenic and marrow inhibition limits their applications (Kumarappan et al., 2007). Therefore the quest for effective anti-cancer drug is an active research field. The search for anticancer agents from plants dates back to 1947, when the cytotoxic properties of podophyllotoxin from Podophyllum peltatum were detected (Kelly,
The discovery of the anticancer properties of vinblastine and vincristine from *Cataranthus roseus* soon followed (Noble *et al.*, 1958) and gave the impulse for wide range of investigations of plant extracts and plant derived compounds for possible anticancer activity. Similar useful drugs like diterpene taxol obtained from plant *Taxus brevifolia* (Suffness, 1987), pyridocarbazole alkaloid ellipticine from *Ochrosia elliptica* and pyrrolo (3,4-β)-quinoline alkaloid camptothecin were obtained from *Camptotheca acuminata* (Hamburger *et al.*, 1991). Branchlets and berries of *Juniperus foetidissima* and *J. sabina* were evaluated by MTT assay against three tumor cell lines (Hela, KB, MDA-MB-468), using ELISA at 540 nm. Extracts of male branchlets of *J. foetidissima* and berries extract of *J. sabina* were cytotoxic against Hela cell line (Hojjat *et al.*, 2009). *Kaempferia galangal*, *Zingiber officinale*, *Piper chaba* and *Mesua ferrea* showed potent cytotoxic activity against Hep-2 cell (Wiratchanee *et al.*, 2010).

**Plants that induce apoptosis**

Apoptosis (programmed cell death) is a highly regulated protective mechanism, through which damaged or superfluous cells are eliminated from the system. Apoptosis is recognized to be vital for normal development, turnover and replacement of cells such as skin cells. Apoptosis can be initiated either at the cell surface (death receptor or extrinsic pathway) or from internal events within the cell (mitochondrial or intrinsic pathway). Both pathways lead to activation of caspases, which are responsible for execution of cell death by cleaving cellular substrates. Extrinsic pathway depends on ligand-activated recruitment of adaptor proteins by death receptors and consequent activation of caspase-8. The intrinsic pathway entails the release of proapoptotic molecules from mitochondria to the cytosol such as
cytochrome c, which then activate caspase cascade. The chief regulators of this pathway are members of the Bcl-2 family proteins (Fig 2).

**Fig. 2** Schematic representation of apoptotic pathways involving p53, Bcl-2 family and caspases. Source: [www.weizmann.ac.il/apoptoticpathways](http://www.weizmann.ac.il/apoptoticpathways)

Additionally, apoptosis of individual cells serves as a defense mechanism against cancer development in an organism by removing genetically damaged or redundant cells that have been inappropriately stimulated to divide by a mitotic stimulus. In fact, apoptosis is deranged in cancer cells, which display reduced propensity towards apoptotic stimuli. Most of the chemopreventive agents have been shown to demonstrate their inhibitory effect through induction of apoptosis.
Role of caspases in apoptosis

Caspases (cysteine-aspartic acid proteases) belong to cysteine protease family and serve as the major effectors of apoptosis. The activation of caspases leads to distinctive morphological changes of cells such as shrinkage, chromatin condensation, DNA fragmentation and plasma membrane blebbing (Degterev et al., 2003). Induction to commit suicide is needed for proper development of organism, to get rid of cells that pose a threat to the organism (e.g. cell infected with virus or cancer or cancer cells), and to remove cells with damaged DNA. Cells undergoing apoptosis are eventually removed by phagocytosis. There are two types of caspases: initiator (apical) caspases and effector (executioner) caspases, both of which are synthesized as inactive proenzymes. Initiator caspases are the first to be activated and include caspase-2, 8, 9 and 10. These in turn cleave and activate effector caspases such as 3, 6 and 7. Effector caspases consecutively cleave, degrade or activate other cellular proteins within the cell triggering the apoptotic process (Boatright, 2003). Caspase inhibitors (Concha, 2002) regulate the initiation of this cascade reaction. Caspase activation can be mediated by intrinsic factors such as Bcl-2 (B-cell lymphoma 2) on the mitochondrial membrane. Bcl-2 is normally found associated with Apaf-1 (Apoptotic protease activating factor 1). Damage to cells causes dissociation of Bcl-2 from Apaf-1 leading to release of cytochrome-c into the cytosol. New complex called apoptosome is formed comprising of cytochrome-c, Apaf-1, and caspase-9. Caspase-9 is cleaved and activates other caspases leading to an expanding cascade of proteolytic activity within the cell (Fig.2). This eventually results in the digestion of structural proteins in the cytoplasm, chromosomal DNA degradation and phagocytosis of the cell. External signals can also affect caspase activation cascade. TNF (Tumor
necrosis factors) and Fas receptors on the cell surface can be triggered upon ligand binding (TNF, Fas, certain toxins and chemicals) to cleave caspase-8 which then initiates increased proteolysis within the cell and its ultimate removal by phagocytosis (Denault, 2002).

**Role of p53 Gene in apoptosis**

p53 acts as a transcriptional activator by triggering transcription of proteins involved in DNA repair (Lohrum, 1999). If the DNA damage is beyond repair, p53 activates apoptotic pathway, which is considered as a last resort to avoid proliferation of cells with mutated DNA (Jin, 2001). Thus, normal p53 function has been demonstrated to be crucial in the induction of apoptosis in humans following DNA damage. This result is further supported by the findings that p53 is the most commonly mutated tumor suppressor gene and found in about 50 - 55% of all human cancers (Malkin, 2001). Lack of p53 function may contribute to the complex network of molecular events leading to tumor formation, as this may allow mutated cells to escape apoptosis. Further, loss of p53 may prompt preneoplastic cells to accumulate additional mutation by obstructing the normal apoptotic response to genotoxic damage (Bode, 2004). In normal cells, p53 protein is latent and highly unstable with a half-life measured in minutes. It is maintained at low levels by targeted degradation mediated by its negative regulator, Mdm2 (Alarcon-Vargas et al., 2002). During DNA damage and/or other stress signals, half-life increases significantly leading to the accumulation of p53 and transcription of target genes such as p21WAF1/CIP1 and Bax. The outcome of this increased transcription depends on cell type, but usually manifested as a very prolonged G1 arrest or apoptosis (El-Diery, 1998). There are several potential mediators of p53-induced apoptosis. The Bax is an apoptosis-
inducing member of the Bcl-2 protein family (Fig.2), whose transcription is directly activated by p53-binding sites in the regulatory region of the gene (Thornborrow et al., 2002). Furthermore, p53 also participates in the initiation of apoptosis by acting directly at mitochondria. Localization of p53 to the mitochondria occurs in response to apoptotic signals and precedes cytochrome-c release and procaspase-3 activation (Haupt et al., 2003).

Apoptosis is vital process for maintenance of homeostasis and eradication of damaged cells. There are many chemopreventive agents, which result in cancer cell death by induction of apoptosis. The methanol extract of Indigofera tinctoria on HCT 116 cells determined by cell viability, DNA fragmentation and comet assay showed that these extracts had an antiproliferative effect on HCT 116 cells via apoptosis (Magesh et al., 2009). Chloroform extract of Epipremnum pinnatum produced significant growth inhibition against T-47D breast carcinoma cells and analysis of cell death mechanisms indicated that the extract elicited both apoptotic and non-apoptotic programmed cell deaths (Lan et al., 2007).

The study of action of willow (Salix safsaf) extract on two types of tumours, Ehrlich ascites carcinoma cells (EACC) and acute myeloid leukemia (AML) showed an increase in DNA fragmentation and expression of p53 protein in tumor cells (Zahran et al., 2005). The cytotoxicity and mechanisms of action of three Hypoxis species on HeLa, HT-29 and MCF-7 cancer cell lines and peripheral blood mononuclear cells (PBMCs) showed.

Plants belonging to Apocynaceae are reported to have anticancer properties. Flavonoids have been shown to possess antimitagenic and anticarcinogenic activity (Kuroda, 1999; Babu et al., 2003). The methanol extract of the fruits of Solanum
*Nigrum* was evaluated for the anticancer activity on the HeLa cell line. The cytotoxicity of *Solanum nigrum* on HeLa cell was evaluated by the SRB (Sulforhodamine B) assay and MTT assay. The methanol extract of these drug showed greater activity on HeLa cell line and little activity on Vero cell line, indicating *Solanum Nigrum* can be used as anticancer agent (Sanjay *et al*., 2009).

The extracts from *Atractylodes lancea*, *Kaempferia galangal*, *Zingiber officinal*, *Piper chaba*, *Mesua ferrea*, *Ligusticum sinense* and *Mimusops elengi* exhibited promising activity against the cholangio carcinoma CL-6 cell line with survival of less than 50% at the concentration of 50 μg. Among these, the extracts from *Atractylodes lancea*, the activity of caspase-7 in two cell lines and DNA fragmentation in all three cancer cell lines (Boukes and Venter, 2011).

A crude aqueous *Sutherlandia frutescens* whole plant extract induced cytotoxicity in neoplastic cells (cervical carcinoma) and CHO (Chinese Hamster Ovary cells) cell lines which was confirmed by DNA fragmentation patterns and flow cytometric analysis (Chinkwo, 2005). A 549 human lung cancer cells exposed to ethanol extract of *Dunaliella salina* showed significant DNA fragmentation (Sheu *et al*., 2008).

Inagaki *et al*., (2007) demonstrated that a compound purified from the ethyl acetate extract of black soyabean vinegar induced DNA fragmentation and the development of apoptotic bodies in U937 cancer cells. *Duchesnea indica* phenolic fraction significantly inhibited SKOV-3 cell proliferation and markedly induced apoptosis by characteristic nuclear DNA fragmentation (Peng *et al*., 2009). Clitocine, a natural biologically active substance isolated from the mushroom *Leucopaxillus giganteus*, induced DNA fragmentation (Ren *et al*., 2008). Agarwala *et al*., (2010)
demonstrated the cytoprotective potential of mangiferin, against mercury chloride induced toxicity in HepG2 cell line using DNA fragmentation as an index. A progressive increase in fragmented DNA was also observed in oesophageal cancer cells (TE-2) treated with the natural antioxidant gallic acid, which was isolated from the fruits of a medicinal Indonesian plant (Faried et al., 2007).

DNA fragmentation was observed in human breast cancer cells treated with cajanol, a novel anticancer agent from pigeonpea (Cajanus cajan) roots. The fragmented DNA ladder in d-gal-treated mice was inhibited by troxerutin, a naturally occurring bioflavonoid (Luo et al., 2010). Ethanol extract of 29 Sophora moorcroftiana seeds significantly inhibited SGC-7901 (gastric cancer) cell proliferation and induced apoptosis by characteristic nuclear DNA fragmentation (Ma et al., 2006).

Akbar et al., (2011) reported the anticancer properties of aqueous extract of Artemisia vulgaris, Cichorium intybus, Smilax glabra, Solanum nigrum Swertia chirayta and curcuma species against various human cancer cell lines and the results showed that these plants cause induction of apoptosis in cancer cells as measured by internucleosomal DNA fragmentation and caspase-3 activation.

Montririttigri et al., (2008) reported the anticancer activity of the pure constituent isolated from Stephania venosa tuber on human ovarian cancer cells (SKOV3). DNA fragmentation and caspase activation studies showed that this plant could significantly inhibit the treated tumour cell proliferation and cause cells death via apoptosis.
**Plant with Antidiabetic activity**

*Diabetes mellitus* is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that this disease affects 25% of the world population. *Diabetes mellitus* is caused by the abnormality of carbohydrate metabolism, which is linked to low blood insulin level or insensitivity of target organs to insulin (Maiti *et al.*, 2004). Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al.*, 2008). Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidemia. Thus the treatment aims to reduce insulin resistance and to stimulate insulin secretion. Diabetes is a metabolic disorder where in human body does not produce or properly use insulin, a hormone that is required to convert sugar, starches, and other food into energy. *Diabetes mellitus* is characterized by constant high levels of blood glucose (sugar). Human body has to maintain the blood glucose levels at a very narrow range, which is done with insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood for the production of energy. Type 1 Diabetes leads to inability to release insulin results in low rates of glucose uptake into muscles and adipose tissue (Lehninger, 2010). Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have
very low side effects. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno botanical information reports about 800 plants that may possess antidiabetic potential (Alarcon et al., 1998). Several herbs have shown antidiabetic activity when assessed using presently available experimental techniques (Jafri et al., 2000). Traditional medicine (herbal) is used for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population (Saravanan et al., 2008).

*Eugenia jambolana* (*E. jambolana*) popularly known as Jamun or Indian blackberry has been indicated in Ayurveda, an ancient system of Indian medicine, for use in DM. In accordance to its claimed anti-diabetic effect in traditional medicine, *E. jambolana* has been reported to have hypoglycemic effects both in experimental models and in clinical studies (Ravi et al., 2004).

Hypoglycemic activity was evaluated in alcoholic extracts of *Coccinia grandis* (*C. grandis*) leaves. Alcoholic extract 600 mg/kg bw was injected orally to mice. Oral administration of alcoholic extract of leaves of *C. grandis* showed significant hypoglycemic effect on blood glucose level in normal fasted rats (Ajay et al., 2009).

It is commonly used spice in various food items in Tamilnadu. *B. juncea* is a traditional medicinal plant, which belongs to family Cruciferae. *B. juncea* aqueous seed extract has a potent hypoglycemic activity, which was investigated in STZ, induced diabetic male albino rat. Doses which have hypoglycemic activity was reported as 250, 350, 450 mg/kg (Thirumalai et al., 2010).
Antidiabetic effect of methanolic bark extract of *Albizia odoratissima* (*A. odoratissima*) in alloxan induced diabetic mice. The methanolic extracts were fed to the animals at a dosage of 250 and 500 mg/kg body weight. The significant reduced in the levels of serum cholesterol, triglycerides, SGOT, SGPT, alkaline phosphatase and decrement of total proteins in alloxan induced albino mice (Dinesh *et al*., 2011).

Antioxidant effect of *Artemisia sphaerocephala* (*A. sphaerocephala*) gum on STZ induced diabetic rat. Levels of serum and liver tissue thiobarbituric acid reactive substances (TBARS) and $\cdot$OH were increased in STZ induced rat. The activity levels of liver and serum superoxide dismutase were decreased. After administration of extract of *A. sphaerocephala*, levels of TBARS and $\cdot$OH were decreased in serum and liver tissue. The significant increments in the levels of liver and serum SOD. *A. sphaerocephala* is very good antioxidant activity (Xin-Zhong *et al*., 2011).

Hypoglycaemic effect of *Berberis vulgaris* (*B. vulgaris*) L. in streptozotocin-induced diabetic rats *B. vulgaris* a traditional medicinal plant which belongs to family Berberidaceae. The results indicated that water extract and saponins shows significant hypoglycemic effect. The serum cholesterol and serum triglycerides levels were significantly increased (Meliani *et al*., 2011).

Diabetes was induced by a single dose of STZ (65 mg/kg) administered by intraperitoneal way. The oxidative stress was measured by tissue MDA. The estimation of pancreas antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). A significant decrement in the levels of pancreas tissue TBARS was recorded in diabetic treated rats when compared to that of normal animals. The activity levels of pancreas antioxidant defense enzymes viz. SOD, CAT, GPx and GST were significantly increased in the diabetic treated
animals. Antioxidant effect of the aqueous leaf extract of *Centaurium erythrea* (Sefi *et al.*, 2011).

**Plant Description**

*Curcuma pseudomontana* J.Graham

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<thead>
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<th>Scientific classification of plant</th>
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<td><strong>Kingdom</strong></td>
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**Vernacular name of Curcuma pseudomontana J. Graham**

**Ayurveda:** Tavaksheera  
**Hindi:** Kachura  
**Marathi:** Raan halada, shindalavana or shindalavani  
**Tamil:** Kattu manjal  
**Malayalam:** Kattu manjal.

*Curcuma pseudomontana* J. Graham belongs to the family Zingiberaceae, commonly known as Hill Turmeric. This species is a rhizomatous herbaceous perennial, which is found in usually moist shady places on the fringes of wet forests or grasslands, in riparian areas, at moderately high altitude along the western side of
the Western Ghats (Mangaly and Sabu 1987). The taxon occurs both in moist deciduous forest and in semi-evergreen forest (Molur et al., 1997). Mycorrhizal associations have been found (Deotare 2003). C. pseudomontana has, small root stock, bearing small almond like or subglobose tubers at the ends of the fibres (but no sessile tubers); tubers pure white inside and it is edible. Leaves are uniformly green, reaching 2ft or more long (including the petiole), 4-6’ broad, lanceolate oblong acuminate, tapering to the base, petioles 8-15 in long. Flowers are bright yellow appearing with the bracts, 2 or 3 in each bract, in autuminal central narrowly oblong spikes 2-5 by 1-1 ¾ inch; peduncles 3-4in long embraced by leaf- sheaths; flowering bract 1 ¼- 1 ¾ by 5/8– 7/8 inch., obovate- lanceolate, the lowest with purple edges only. The inflorescence of C. pseudomontana is lateral in the early part of the rainy season and terminal later in the season. The colour of the coma is variable within the species (Mangaly and Sabu 1987). Flowering starts from the month of June and ends in the month September. Curcuma is a taxonomically difficult genus and problematic for plant hunters, herbarium technicians as well as taxonomists (Mangaly and Sabu 1993).

Use of Curcuma pseudomontana J. Graham in Folk Medicine

C. pseudomontana rhizome is beneficial against leprosy, dysentery, cardiac diseases (Yoganarasimhan, 1996). Jatapu and Kaya tribes apply warm tuber paste to treat body swellings. Khand tribes apply the tuber paste on the head for cooling effect, crushed and boiled rhizome is edible (Patil et al., 2000). Women of Jatapu and Savara tribes eat boiled tubers to increase lactation (Ramarao et al., 2000). The Kukus-Mukus eat fresh tubers as a blood purifier (Bhosle et al., 2006). Rhizome past used to apply to wounds and cuts (Sudhakar et al., 2009). The Savara, Bagata, Valmiki tribes of
Andhra Pradesh use tuber extracts to cure jaundice and Bagata tribes use this plant for Diabetes (Padal et al., 2012). The tubers are also edible (Acharya, 2012).

Therefore based on the literature survey and available data, the rhizome of *Curcuma pseudomontana* J.Graham was selected in present study to evaluate the pharmacognostic analysis, antimicrobial, antituberculosis, antioxidant, anticancer and antidiabetic activity.