1.0 INTRODUCTION

1.1 Medicinal plants – History and context

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta et al., 2005; Sandhu and Heinrich, 2005).

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie et al., 1996), together with their side effects and lack of healthcare facilities (Griggs et al., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu et al., 1999; Idu et al., 2007; Mann et al., 2008; Ammara et al., 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari et al., 1986; Van Wyk et al., 2000).
Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell et al., 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava, et al., 1996; Mahesh and Sathish, 2008).

1.2 Natural antibiotic properties of plant secondary metabolites

The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids, carbohydrates etc.).
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). From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink et al., 2005).

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 micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities 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 (e.g. 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.

Some biologically active plant compounds have found application as drug entities or as model compounds for drug synthesis and semi-synthesis.
A survey of current pharmaceutical use revealed that, of the total prescription drugs dispensed, 25% are plant derived (Farnsworth and Morris, 1976; Ogundipe et al., 1998). Plant compounds are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. However, there is an increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic micro-organisms build against the antibiotics (Essawi and Srour, 1999). New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants (Cox, 1994). Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of indigenous medical practices (Gilani and Rahman, 2005). Eloff (1999) stated that the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains.

Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design (Wink et al., 2005).
1.2.1 Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste (Manske, 1965; Guillermo and Victor, 1999).

1.2.2 Flavonoids

Flavonoids are derived from 2-phenylchroomen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature’s biological response modifiers because of their inherent ability to modify the body’s reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Rauha et al., 2000; Cushnie and Lamb, 2005; Filippos et al., 2007; Spencer and Jeremy, 2008).

1.2.3 Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts; leaves,
stems, roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently (Oakenfull and Sidhu, 1990). Saponins also inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells. Saponins are the plant’s immune system acting as an antibiotic to protect the plant against microbes and fungus (Shideler, 1980; Chatterjee and Chakravorty, 1993).

1.2.4 Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes and are also used as a laxative (Chatterjee and Chakravorty, 1993; Samp, 2008)

1.2.5 Cardiac glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as
secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos et al., 2007).

1.3 Antimicrobial activity of plants

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali et al., 1998; Barbour et al., 2004; Yasunaka et al., 2005). Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Ibrahim, 1997; Towers et al., 2001; Koshy et al., 2009). Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Murray, 1992; Madunagu et al., 2001; Koshy et al., 2009; Senthilkumar and Reetha, 2009) Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to
continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re-emerging infectious diseases (Ikenebomeh and Metitiri, 1988; Rojas et al., 2003; Geyid et al., 2005).

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Paz et al., 1995; Vlietinck et al., 1995; Kudi et al., 1999; Palambo and Semple, 2001). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Onwuliri and Dawang, 2006; Mahesh and Sathish, 2008).

The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Eloff, 1999). The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs (Barbour
et al., 2004). There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials (Barbour et al., 2004).

1.3.1 Role of Antibiotics in bacterial treatment

Antibiotics are the mainstay of bacterial treatment (Archer and Ronald, 2001). The goal of these drugs is to kill the invading bacteria without harming the host. Antibiotic effectiveness depends on mechanism of action, drug distribution, site of infection, immune status of the host, and resistance factors of bacteria (Archer and Ronald, 2001; Roden, 2004).

Antibiotics work through several mechanisms. Some (such as vancomycin and penicillin) inhibit formation of bacterial cell walls. Erythromycin, tetracycline, and chloramphenicol interrupt protein synthesis. Still others inhibit bacterial metabolism (sulfa drugs) or interfere with DNA synthesis (ciprofloxacin, rifampin) and/or cell membrane permeability (polymyxin b) (Conte et al., 2002).

When antibiotics were discovered in the 1940s, they were incredibly effective in bacterial infection treatment. Over time, many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance (Perez et al., 1990; Barie, 1998; Domin, 1998; Okeke et al., 2005). Bacteria may be naturally resistant to different classes of antibiotics
or may acquire resistance from other bacteria through exchange of resistant genes. Indiscriminate, inappropriate, and prolonged use of antibiotics have selected out the most antibiotic-resistant bacteria (Van Waaig and Nord, 2000; Petrosillo and Pantosti, 2002). Antibiotic-resistant strains have emerged in hospitals, long-term care facilities, and communities worldwide (Flaherty and Weinstein, 1996; Jacobs, 1999; Levin and Levy, 2003).

1.3.2 Human Pathogenic Microbes

Microorganisms are very diverse and even though their different cells look similar in morphology and produce similar colonies, it becomes necessary to identify the organisms by their biochemical characteristics that help to properly classify the organisms, causing diseases that kill people, animals and plants (Table 1).

*Staphylococcus aureus* is a common coloniser of human skin and mucosa. *S. aureus* can cause disease, particularly, if there is an opportunity for the bacteria to enter the body. Prescott *et al.*, (2005) states that *S. aureus* is the most important human staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, toxic shock syndrome amongst other diseases. *S. aureus* is also a pathogen frequently reported to produce food poisoning, which leads to cramps and severe vomiting. Most strains of this bacterium are sensitive to many antibiotics, and infections can be effectively treated (Abbas *et al.*, 2004).

*Pseudomonas aeruginosa* is an opportunistic pathogen and exploits some break in the host defenses to initiate an infection. It is a common
environmental microorganism present in water and soil and is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen (Prescott et al., 2005). The bacterium is naturally resistant to many antibiotics due to the impermeability characteristics of the outer membrane. Moreover, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics (Craig, 1997; Okemo et al., 2001).

**Bacillus subtilis**, is a food-poisoning, Gram-positive, facultative, aerobic, sporulating bacteria normally found in soil. *B. subtilis* is normally considered as being non-pathogenic; but it has been linked to food-borne illnesses, causing diarrhoea, nausea, vomiting, and associated with rice dishes served in oriental restaurants and its infection is self-limiting (Willey et al., 2008). *B. subtilis* produces subtilisin, which is an extracellular enzyme that catalyzes the breakdown of proteins into polypeptides, resembles trypsin in its action, and has been shown to be a potent occupational allergen (Willey et al., 2008).

**Escherichia coli** are usually found in the gastro-intestinal tracts of warm blooded organisms. The most common cause of urinary tract infection in humans is *E. coli*, causing at least five types of gastro-intestinal diseases in humans. Pathogenicity is generally due to the presence of one or more virulence factors, including invasiveness factors, heat-labile and heat-stable enterotoxins, verotoxins and colonization factors. Pathogenic strains are usually identified by detection of specific virulence factors or of a serotype associated with a virulence factor (Willey et al., 2008).
*E. coli* is an emerging cause of food-borne infection which leads to bloody diarrhoea and occasionally to kidney failure. Most cases of the illness have been associated with eating under-cooked, contaminated, ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission if hygiene is inadequate. *E. coli* infection can also occur after drinking raw milk and after swimming or drinking contaminated water (Akinnibosun *et al.*, 2008).

*Klebsiella pneumoniae* is a Gram-negative, non-sporulating, facultative, aerobic shaped rod bacterium that is normally found in the human gastro-intestinal tract. An adhesion to a mucosal surface is often the first step in the development of an infection. A survey of the presence of *Klebsiella* in urban residents, hospital personnel, and newly admitted patients showed that 30-37% of individuals carried *Klebsiella*, including a 29-35% faecal carriage and a three-to-four-percent throat carriage. Strains of *K. pneumoniae* and *K. oxytoca* which have not acquired any resistance are determined as naturally resistant to ampicillin and carboxy penicillin but susceptible to other beta-lactam antibiotics. This is due to the production of a chromosomal penicillinase which is inhibited by clavulanic acid (Cohen and Powderly, 2004).

*Shigella sonnei* is a non-motile, rod shaped nonspore-forming, facultative anaerobic Gram-negative and lactose-fermenting bacterium. *S. sonnei* is extremely fragile in experimental settings. Its natural habitat is a low pH environment such as the human gastrointestinal tract. Its optimal environmental temperature is 37º C, similar to the temperature in the human
body. Therefore, human’s gastrointestinal tract appears to be the only found natural host of *S. sonnei* known so far. *S. sonnei* causes an enterobacterium disease called Shigellosis. *S. sonnei* is spread mostly by means of faecal-oral transmission. Other possible modes of transmission can be from ingestion of contaminated food or water, and subcutaneous contact with inanimate objects and, most rarely, sexual contact. Food prepared by the contaminated person may easily become contaminated with *Shigella* bacteria (Yang and Yang, 2005).

*Proteus vulgaris* is a rod-shaped, Gram negative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water and fecal matter. It is known to cause urinary tract infections and wound infections. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation, and those whose infections were acquired in the hospital have an increased frequency of infection caused by *Proteus* and other organisms (eg, *Klebsiella, Enterobacter, Pseudomonas, Enterococci, Staphylococci*) (O'Hara et al., 2000).

*Candida albicans* is the most common organism implicated in fungal infections, which is found in the human digestive tract, mouth, and genital region (Eggiman and Garbino, 2003). Under normal circumstances, levels of *Candida* are controlled by beneficial bacteria. However, if the bacteria-fungus balance is upset, by the use of antibiotics or if the immune system is compromised, an overgrowth of *Candida* can occur, resulting in infection (Braunwald and Kasper, 2001).
Fungal overgrowth is encouraged by certain pH levels and the availability of sugar (Howard and Gordon, 1995; Buddington and Williams, 1996; McGinnis and Molina, 1996). People with the right conditions for fungal infection, such as a high sugar diet, are at higher risk. Also, *Candida* infections can be spread to vulnerable people with depressed immune systems who are in the hospital, where the fungus is commonly found on the hands of care givers.

*Cryptococcus neoformans* yeast lives in both plants and animals. *C. neoformans* grows as a yeast (unicellular) and replicates by budding and does not exist in a hyphae or pseudohyphae form. *C. neoformans* causes lung infection. However, fungal meningitis, occur as a secondary infection for AIDS patients. Infections with this fungus are rare in those with fully functioning immune systems (Saag et al., 2000).

## 1.4 Antioxidant potential of medicinal plants

In living systems, oxidation is a basic part of the normal metabolic process, in which Reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated (Finkel and Holbrook, 2000; Halliwell, 2000; Pietta, 2000; Vijayabaskaran *et al.*, 2010). Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging (Mclarty,
Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body.

There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, (Zheng and Wang 2001; Cai et al., 2003; Govindarajan et al., 2005; Naruthapata and Supaporn, 2009). So to maintain a healthy body, one should always increase the intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above disease conditions (Halliwell, 1994; Klipstein et al., 2000; Bimal et al., 2011).

Naturally occurring antioxidants can be used in foods and also for prevention and treatment of free radical-related disorders (Middleton et al., 2000; Kumar and Kumar, 2009) which can also be replaced by commercially available, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are quite unsafe to use and is restricted due to their carcinogenic effects (Velioglu et al., 1998; Vinay et al., 2010). Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and
regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Shreejayan and Rao, 1997; Hagerman et al., 1998; Balakrishnan et al., 2009).

The most commonly used methods for measuring antioxidant activity are those which involve the generation of free radicals which are then neutralized by antioxidant compounds. DPPH is a well-known radical and a trap ("scavenger") for other radicals (Husain et al., 1987, Visioli et al., 2000; Parr et al., 2004; Solai et al., 2010). Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm. DPPH method measures electron donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen will react with DPPH, thus neutralizing its colour from a deep purple to a light yellow by electrons from the oxidant compounds. The concentration of DPPH at the end of a reaction will depend on the concentration and the structure of compound being scavenged (Naik et al., 2005; Balasundram et al., 2006; Masoko, 2007).
1.5 Importance of plants in anticancer activity

Cancer is one of the most life-threatening diseases with more than 100 different types. Due to lack of effective drugs, expensive cost of chemotherapeutic agents and side effects of anticancer drugs, cancer can be a cause of death. Cell death can occur through several different mechanisms, of which the most widely described are apoptosis and necrosis (Sengupta et al., 2004; George et al., 2010). A significant physiological consequence of cell death by apoptosis is that the apoptotic cells are immediately phagocytosed by macrophages. Therefore, the release of intracellular molecules that cause secondary disturbance to the surrounding tissue is limited to a low level compared with necrosis, which causes further tissue destruction and inflammation (Cohen, 1993; Earnshaw, 1995). Now people have started realizing the importance of natural bioactive substances found in fruits, vegetables, and herbs, as antioxidants and functional foods (Wang et al., 1997; Kitts et al., 2000; Lee and Lim, 2001). Some of these substances are believed to be potential chemopreventive or therapeutic agents for cancer (Pezutto, 1997; Christou et al., 2001; Mukherjee et al., 2001). Most of these substances exert their chemotherapeutic activity by blocking the cell cycle progression and triggering apoptotic cell death. Therefore, the induction of apoptosis in tumor cells has become an indicator of the tumor-treating ability of naturally derived bioactive substances (Smets, 1994; Paschka et al., 1998).

Apoptosis or programmed cell death is a highly organized physiological process to eliminate damaged or abnormal cells. It also plays
a major role in embryogenesis where apparently normal cells undergo apoptosis. It is involved in maintaining homeostasis in multicellular organisms (Perandones et al., 1993). During the past decades, the induction of apoptosis in cells has been recognized as a novel strategy for the identification of anticancer drugs (Smets, 1994; Watson, 1995; Panchal, 1998). Apoptosis itself also plays an important role in the development of various diseases including cancer (Fisher, 1994; McConkey et al., 1996). Apoptosis are triggered by activation of the death receptor (extrinsic) and mitochondrial (intrinsic) pathways, results from activation of members of cysteine protease family called caspases (Miller, 1999; Wolf et al., 1999; Fan et al., 2005). Mitochondria are involved in a variety of key events, including release of caspase activators, changes in electron transport etc. (Zamzami et al., 1996; Green and Reed, 1998; Gottlieb, 2000). Alterations in mitochondrial structure and function have been shown to play a crucial role in caspase-9-dependent apoptosis (Green and Kroemer, 1998) by releasing apoptotic factors from mitochondria including cytochrome C. In this manner, released cytochrome C interacts with Apaf-1 and pro-caspase-9 to form the apoptosome. Then caspase-9 cleaves and activates caspase-3, the executioner caspase, which cleaves poly (ADP-ribose) polymerase (PARP) and activates endonucleases leading to DNA fragmentation (Fig 2) (Cai et al., 1998; Cecile et al., 2004).

In addition to monitoring caspase activity, some of the biochemical features of apoptosis such as loss of membrane phospholipid asymmetry and DNA fragmentation can also be used to identify apoptosis (Williamson,
The outstanding feature of apoptosis is its remarkable stereotyped morphology showing cytoplasmic condensation, plasma membrane blebbing and nuclear pycnosis, leading to nuclear DNA break (Fig 3) down into multiples of ~200bp oligonucleosomal size fragments (Kerr et al., 1972; Telford et al., 1991; Williamson, 2000). In addition, apoptotic cells cultured in vitro will eventually undergo "secondary necrosis". After extended incubation, apoptotic cells ultimately shut down metabolism, lose membrane integrity and release their cytoplasmic contents into the culture medium (Riss and Moravec, 2004). Therefore, cells that have initiated apoptosis may exhibit some of the morphological phenotypes associated with necrosis.

The accepted modality for cancer treatment involves surgery, radiation and drugs, singly or in combination. Cancer chemotherapeutic agents can often provide temporary relief from symptoms, prolongation of life and occasionally leads to a cure. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal situation is achievable by inducing apoptosis in cancer cells. The life span of both normal and cancer cells is significantly affected by the rate of apoptosis. Thus, modulating apoptosis may be useful in the management and therapy or prevention of cancer. Synthesis or modification of known drugs continues to be an important aspect of research.

Recent studies on tumor inhibitory compounds of plant origin have yielded an impressive array of novel structures. Epidemiological studies suggest that consumption of diets containing fruits and vegetables, which
are major sources of phytochemicals and micronutrients, may reduce the risk of developing cancer. Certain products from plants are known to induce apoptosis in neoplastic cells but not in normal cells (Chiao et al., 1995; Hirano et al., 1995; Jiang et al., 1996; Shaikh et al., 2011). It has become increasingly evident that apoptosis is an important mode of action for many anti-tumour agents, including ionizing radiation (Radford et al., 1994), alkylating agents such as cisplatin and camptothecin. Camptothecin has been effective against a broad spectrum of tumours. Camptothecin is a quinoline based alkaloid found in the bark of the Chinese camptotheca tree. It has been used for psoriasis, leukemia and diseases of liver, gall bladder, spleen and stomach.

Even though there are number of synthetic antitumour agents available, efforts are still on to search for effective naturally occurring anticarcinogens that would prevent, slow or reverse cancer development. Plants have a special place in the treatment of cancer. It is estimated that plant derived compounds constitute more than 50% of anticancer agents (Newman et al., 2003; Nipun et al., 2011). Cell viability assays can be combined with apoptosis assays to provide more information about mechanisms of cell death through multiplexing assays on a single sample (Dive et al., 1992).

1.6 Medico-Ethno botanical survey

Members of the Sansevieria genus are commonly referred to as bowstring hemp, piles root in English. Sansevieria has undergone many changes with regard to classification. Initially, this genera was classified as
part of the *Liliaceae* Family (Watt *et al*., 1962), whereas later documentation has categorized it under the *Agavaceae* Family (Mimaki *et al*., 1996a). The most recent classifications state that *Sansevieria* forms part of the *Dracanaceae* Family (Germishuizen and Meyer, 2003; Mbugua and Moore, 2003). These plants are popular garden and indoor plants due to their ability to flourish under low light conditions and need little growth attention. It is a native of tropical Asia, now pantropic in cultivation.

*Sansevieria roxburghiana* is a xerophytic herbaceous to shrubby succulent perennial plant with evergreen strap-shaped leaves, growing to 20 cm to 3 m tall, often forming dense clumps from a spreading rhizome or stolons (Plate 1). The flowers are greenish-white, produced on a simple or branched raceme 40-90 cm long. The fruit is a red or orange berry (Reed, 1978).

**Taxonavigation**

- Kingdom – Plants
- Subkingdom – Tracheobionta
- Superdivision – Spermatophyta
- Division – Magnoliophyta
- Class – Liliopsida
- Subclass – Liliidae
- Order – Liliales
- Family – Dracanaceae
- Genus – Sansevieria
- Species - *Sansevieria roxburghiana*
  Schult. & Schult. f.
  (Indian bowstring hemp)
1.6.1 Medicinal properties

The medicinal uses of *S. roxburghiana* include treatment for abdominal pains, earache, diarrhoea and hemorrhoids traditionally, in treating ear aches and hemorrhoids, the leaf of this plant is heated and the warm juice is squeezed onto the affected area.

The roasted leaves of *S. roxburghiana* are used as an emollient. Root stocks are used as cough medicine in India. Juice of tender shoot is administered to children to clear their throats to clear phlegm, as a febrifuge, tonic and purgative. In India, tender roots and rhizome are used as expectorant and in bone setting (Reddy *et al*., 2006). In viral diseases associated with nasal discharge, slightly warmed leaf juice is used as nasal drops (Joy *et al*., 2008).

The medicinal properties of *Sansevieria* species is well documented (Wasicky and Hoehne, 1951; Mimaki *et al*., 1996a; Mimaki *et al*., 1996b). Some species have an ethnopharmacological background, in particular *S. trifasciata* which in South Africa and tropical America is used for the treatment of inflammatory conditions and sold as a crude drug in the market to treat victims of snakebite (Morton, 1981). In Nigeria, the leaves and roots of *S. liberica* are used in traditional medicine for the treatment of asthma, abdominal pains, colic, diarrhoea, eczema, gonorrhea, hemorrhoids, hypertension, menorrhagia, piles, sexual weakness, snake bites and wounds of the foot (Gill 1992; Osabohien and Egboh, 2008; Adeyemi *et al*., 2009). *Sansevieria* plays a major role in the fibre industries as it is a source of leaf fibre for making strong cords (Watt *et al*., 1962).