CHAPTER-II

REVIEW OF LITERATURE
Cancer

Cancer is a disease that displays uncontrollable growth beyond the normal limit and spread into surrounding tissue (Moss, 2004). Mutation event in the genes is the critical for cancer development. Cancer is the second most common cause of death in the world after heart disease and is expected to overtake it by the end of year 2015 (Science Daily Magazine, 2008). A normal cell suddenly turns into a cancerous cells and starts dividing continuously without check, leading to the development of solid clumps (tumors). It has been reported that cancer originates in our own cells, there are several factors and both intrinsic and extraneous factors are responsible for the cancer development.

Several biological factors (intrinsic) like age and hormonal status, family history. External factors like diet, alcohol, tobacco use, radiation, viruses like human papiloma virus, Epstein-Barr virus Hepatitis B virus are reported to cause cancer. Reactive oxygen species (ROS) and other free radicals are produced in the body both during the normal metabolic process and as well as by the interaction of the external agents, these free radicals interacts with DNA and produce gene mutations and chromosomal aberrations leading to cell transformation, free radicals are considered as major roles in the induction of cancer (Clayson et al., 1994).

Cancer is a multistep process, the concept of multistage carcinogenesis initiation, promotion and progression. Initiation is irreversible changes in the somatic cells that arise spontaneously in somatic cells; this is considered to be the first step in carcinogenesis cellular genome under mutations. Five different mechanisms are considered for proto oncogene activation. Progression is the process that gives increasing number of malignant sub populations, cells that transform into a state in which they are committed to the malignant development some more gene mutations accumulates during the development (Fishe1 and Kolodner, 1995). Tumor metastasis involves cell-cell and cell- matrix interactions involving specific cell surface molecules, this is influenced by cell adhesion molecules and cadhreins is one among them. Growth of the tumor depends on supply of growth factors and removal of toxic
molecules, therefore for tumor mass depends on adequate blood supply though blood capillaries, this called as tumor angiogenesis.

Among the various types of cancer, breast cancer is the most common form of cancer and a leading cause of cancer mortality among the women. Incidence of breast cancer is rising in India and is the second most common cancer diagnosed in women after cervical cancer. Breast cancer incidence has age specific, rapid rate of increase before menopause (age 40-50) slows down after that. Numbers of risk factors like family history of breast cancer, age, diet, body mass, environmental toxins, lactation, hormone imbalances, implant, etc. are the major risk for breast cancer (Wolk et al., 1998).

**Treatment**

In recent years, chemotherapy, radiation therapy and surgery have been the major attempts of cancer treatment and continue to be very important. Chemotherapy involves the use of cytotoxic drugs to treat the cancer cells. The major drawback of chemotherapy is the cytotoxic effect on normal cells of the body. Usually a combination of all the three treatments is employed for any given cancer (Schatzlein, 2006). Major challenges in cancer chemotherapy are cytotoxic effect on normal cells and multidrug resistance against anticancer agents (Carelle et al., 2002); these chemotherapeutic drugs exert side effects to the patients. For these reasons researches and development of a new class of anticancer agents which exhibit efficient and selective toxicity to tumor cells is attracting increased attention.

**Endophytes**

Endophytes are the microorganisms that reside inside the plants, earliest record of mutulistic symbiosis was observed in the roots of the fossil tree Amyelon radicians from the Paleozoic era (Bacon and Hill, 1996). It has been considered important not only from the standpoint of original of endophytic symbiosis, but also for evidence of plant-fungus association. First evidence of isolation of endophytes was from the seeds of Lolium temulentum (Wilson, 1994).
Endophytic term has been defined in various ways by different workers. Endophytes are any organism occurring within plant tissue. Mutualist, those colonizes in living plants and do not cause symptoms of disease at any time of their life cycle. Bacteria and fungi as endophytes which invade living plants throughout all parts of their life cycle and causes asymptomatic infections, without causing disease (Wilson, 1994). The definition was simplified as microorganisms residing inside plants without producing visible symptoms were categorized as endophytes (Azevedo et al., 2000). The definition describes about endophytes by authors is equally applicable to bacterial endophytes (Stone et al., 2000).

The plant species on this earth harbor endophytes (Arnold and Herre, 2003). Among endophytes bacteria and fungi, endophytic fungi are a polyphyletic group of highly diverse, primarily ascomycetous fungi by their occurrence within asymptomatic tissues of plants, are found in above ground tissues of liverworts, hornworts, mosses, lycophytes, equisetopsids, ferns and seed plants from the arctic to the tropics, and from agricultural fields to the most poetically diverse tropical forests (Arnold, 2007). There may be one million species of endophytic fungi alone. Most common endophytic fungi observed were Aspergillus, Phomopsis, Wardomyces, Penicillium and unidentified fungus (Rodrigues, 1994).

Diversity and distribution of endophytes

Endophytes are ubiquitous distributed in all plants growing in tropical, temperate and boreal forest with the host ranging from herbaceous plants to woody trees in various habitats, including extreme arctic alpine and xeric environments to mesic temperate and tropical forests. Pioneering work done by the different workers worldwide on the detection, taxonomy, species composition, distribution, biological, ecological and physiological aspects of endophytes (Johnson and Whitney, 1989).

Endophytic fungi from aerial tissue are documented from conifers (Wang et al., 2007) and from grasses (Bacon et al., 1977). Further, the presence of bacterial and fungal endophytes are reported from marine algae, mosses, ferns, angiosperms, gymnosperms, broad leaved trees, estuarine plants, diverse herbaceous annuals,
deciduous and perennials. Similarly, studies on diversity of endophytes have been carried out in the northern hemisphere. Reports on endophytes from tropics include those from Bermuda (Southcott and Johnson, 1997), Hong Kong (Brown et al., 1998), Brazil (Rodrigues and Samuels, 1999), Thailand (Bussaban et al., 2001) and Guyana (Cannon and Simmons, 2002).

India is the largest collection of medicinal plants and is rightly called the “Botanical garden of the world”. Although studies on endophytes have been carried out since two decades, only around 120 host plants belonging to 75 families have been screened for their endophytic assemblages. In India studies on endophytic fungi has been focused on medicinal plants (Gautam et al., 2013) was screened to isolate endophytic fungi from aerial parts of the plant. Studies on endophytes of roots were considered differently, attempts were made to isolate root endophytes from different hosts (Seena and Sridhar, 2004). Rich fungal diversity was observed from different medicinal herbs, they observed some endophytes were common to the entire host and few appeared to be host specific (Rajagopal et al., 2010).

Influence of ecological factors on the diversity of endophytes, the relationship between geographic locations and seasonal variation has been reported (Verma et al., 2007). Biodiversity and tissue recurrence of endophytic fungi in Tripterygium wilfordii, few endophytes were isolated from the twig of xylem and bark, but not isolated from the roots, leaves and flowers, the study concludes that species composition and frequency of endophytic species were dependent on tissue type.

Endophytic fungal diversity in young, old and senescent leaves of Ocimum basilicum L. A medicinal plant has been reported, the study provides the first report on the diversity of endophytic fungi of medicinal plants from southern India (Gangadevi and Muthumary, 2007). There were qualitative and quantitative changes in the endophytic diversity were observed in endophytic mycoflora of neem from different regions and plant parts. Similarly endophytes of leaf stem and root tissue of Catharanthus roseus collected from two different ecosystems in North India, it suggests a fungal host and tissue specificity had a little variation in their species richness (Kharwar et al., 2008).
Tissue, organ and host specificity and host-endophytes interaction

Most of the endophytes are recovered from the aerial parts of the host plant, but there is some research finding that endophytes are organ and tissue specificity. Organ specificity of endophytic fungi was observed in wheat plants. A similar report was observed in *Picea abies*, Norway spruce and White Fir. It has been reported that the organ specificity of endophytic fungi is due to adaptation to particular micro ecological and physiological conditions present in the particular organ. Tissue specificity is exhibited by endophytes is also reported as a result of their adaptation of plant to different physiological conditions (Rodrigues and Samuels, 1999).

Fungal endophytes from five medicinal plant species, reported 18 species of endophytic fungi from bark, stem and leaf segments, highest species richness as well as colonization of endophytic fungi was found in the leaf segment rather than the stem and bark segments of the host plant species. The study provides evidence that fungal endophytes are host and tissue specific. Apart from tissue specificity endophytic fungi have also shown host specificity. Endophytic fungal communities from *Pinus sylvestris* and *Fagus sylvatica* growing at the same site were observed.

Endophytes residing inside the plant enhance growth of host plants by producing phytohormones without any apparent facilitation of host nutrient uptake (Schulz and Boyle, 2005). Endophytic mycelial extract of *P. fortinii* induced a similar increase in *Larix decidua* shoot and root biomass, growth promotion was due to indole acetic acid (IAA) a hormone synthesized by the endophytic fungus *in vitro*. This provides the evidence of endophytes in growth enhancement.

Endophytes help the host plants to tolerate and withstand drought, salts and high temperature. Endophytic fungi provided protection to their host plant from pathogens and from pest was reported (Akello *et al.*, 2007). A similar effect was observed in *Piriformospora indica* to induce resistance to fungal diseases and tolerance to salt stress in barley (Waller *et al.*, 2005). It has been reported that systemic alternation was associated with increase of anti-oxidative capacity due to an activation of the glutathioneascorbate cycle and results in an overall increase in grain yield. Hence,
such symbioses are of great importance, since they might help plants to adapt to global climate change.

Endophytic fungi residing in the host plant can reduce infection by producing alkaloids i.e. toxic to insects and vertebrates. Leucinostatin A is especially active against the oomycetous plant pathogenic fungus, *Pythium ultimum* (Strobel *et al.*, 1996). It has been reported that mechanisms of endophyte-induced resistance is related to the nutritional status of the host, and to increase the fitness of plants by enhancing their tolerance to abiotic stress.

Studies reported that endophytic potential in host protection to biotic and biotic factors, endophytes improve host resistance to pathogens by different mechanism (Mandyam *et al.*, 2010), competition between endophytes and pathogen for the same source, ability of endophytes to enhance the host to produce phytoalexins, biocidal compound, or ability of the endophytes itself to produce antimicrobial agents (Rai *et al.*, 2001), improving the host resistance to pathogens by inducing host defense response by localized endophytes (Gianinazzi *et al.*, 1996).

**Natural products from endophytes**

Endophytes are gaining importance because of their enormous potential to produce bioactive compounds. The discovery of taxol a potent anticancer drug from endophytic fungis *Taxomyces andreane* of yew plant *Taxus brevifolius* attracted attention of drug discovery (Stierle *et al.*, 1993). Endophytic fungi isolated from *Taxus baccata* produced Leucinostatins compound, which acts as anti-fungal and anti-cancer agent. The amount of bioactive compounds produced by endophytic fungi is relatively small when compared with host tree. However, the short generation time and high growth rate of fungi make worthwhile to examine these species for the production of valuable drugs. Endophytes *Phoma* sp. isolated from *Taxus wallichinia* had reported two metabolites as Altersolanol A and G-hydroxy- 6-methyl benzoic acid, which were able to produce anti-microbial activity (Strobel *et al.*, 1996).

Endophytes are the abundant, dependable source of biological active compounds with potential exploitation in pharmaceutical and agriculture field (Strobel and Daisy,
2003). They have been characterized to produce novel antibiotics, antiviral, immunosuppressants, anticancer compounds, antioxidant and other bioactive compounds. It has been reported that natural compounds produced by endophytes have been promising potential usefulness in safety and human health concerns, there is an significant demand of drug industry (Strobel et al., 2004).

Schulz et al. (2002) have isolated 6500 endophytic fungi and tested their biological potential, around 135 secondary metabolites were found among them 51% of them are bioactive compounds, they concluded that endophytic fungi are a good source of novel compounds. Bioactive compound isopestacin obtained from the endophytic fungus Pestalotiopsis microspora, compound was isolated from the cultured broth of fungus, compound was crystallized and structure was determined by X-ray crystallography and shows that compound posses’s antifungal and free radical scavenging activity (Strobel et al., 2002).

Antimicrobial, anticancer and antimalarial compounds have been isolated from endophytic fungi of Thai medicinal plants, out of 81 Thai medicinal plant species, 360 morphologically distinct fungi were reported and tested for biological active compounds. 40 isolated compounds showed the antiviral activity against Herpes simplex virus, anticancer activity against human oral epidermoid carcinoma cells and breast cancer cells, it was observed that Thai medicinal plants inherits diverse endophytes possessing a potential source of bioactive compounds (Wiyakrutta et al., 2004). Some unknown compounds showing significant bio-activity has been extracted from an endophytic fungus found in the leaves of the plant Desmodium uncinatum from the islands of Papua New Guinea. Endophytic extracts showed antibacterial and anticancer activityto destroy HeLa cervical cancer cells (Owen et al., 2000).

A potent inhibitor of HIV-1 protease, hinnulquinone was isolated from the endophytic fungi from the leaves of Quercus cocciifera, research showed that endophytic fungi are the potential synthesizers of hinnulquinone (Singh et al., 2004). Bioactive compounds 2-hexyl-3-methyl-butanedioic acid and cytochalasin D isolated from the endophytic fungus Aylaria sp. from Brazilian Cerrado, exhibited strong antifungal activity (Araujo et al., 2002).
Endophytic fungi isolated from *Acanthus ilicifolius* and *Acrostichum aureum* of southwest coast of India showed its anti-microbial property against *B. subtilis*, *Enterococcus* sp., *K. pneumoniae*, *P. aeruginosa*, *S. typhi* and *S. aureus* (Maria et al., 2005). Citrinin (polyketide) produced by endophytic fungus *Penicillium janthinellum* from fruits of *Melia azedarach*, reported antibacterial activity against *Leishmania* sp. (Marinho et al., 2005).

Endophyte *Bacillus amylobiophacien* isolated from Chinese medicinal plant *Scutellaria baicalensis gerogi* was found to produce cyclic lipopeptides, surfactins and fengycins with broad spectrum antibacterial and antifungal activities. Endophyte *Xylaria* sp. isolated from the medicinal plant *Ginkgo biloba*, methanolic extract exhibited strong antioxidant capacity due to the presence of phenolics and flavonoids compounds (Liu et al., 2007).

In India attempts were made to isolate taxol with potent cytotoxic action from two endophytes *Colletotrichum gloeosporioides* and *Bartalina robillardoides* Tassi from *Justicia gendarussa Burm F.* and *Aegle marmelos*, respectively (Gangadevi and Muthumary, 2007). Endophytic *Xylaria* sp from twig of *Ginkgo biloba*, reported to produce 7-amino 4-methylcoumarin, compound that showed strong antibacterial and antifungal activities (Liu et al., 2008). Secondary metabolite obtained from the endophytes was reported to inhibit the growth of human pathogenic bacteria (Nithya and Muthumary, 2011).

Antimicrobial potentials of endophytic fungi inhabiting ethno medicinal plants of Simililpal biosphere reserve India, around 60 fungal endophytes belonging to 14 genera were isolated out of which 31 endophytes were in filamentous forms and 29 of them as yeast colonies. Species of *Curvularia, Fusarium, Alternaria* and *Penicillium* were isolated as dominat and host specificity. Endophytic extract displayed both antibacterial and antifungal activity on all test pathogens, studies concluded that endophytes of ethno medicinal plants could be promising source of antimicrobial substance. Further two new chlorinated benzophenone derivatives, Pestalachlorides A and B, reported from endophytic fungus *Pestalotiopsis adusta*, exhibited significant
antifungal activity against three plant pathogenic fungi, *Fusarium culmorum*, *Gibberella zeae* and *Verticillium albo-aetrum* (Jiang et al., 2008).

Endophytic secondary metabolites steroids, iridoid have been reported by endophytes of ethomedicinal plants of Jammu and Kashmir (Ahmed et al., 2013). Similarly secondary metabolites of endophytic fungal extract from the leaves of medicinal plant *Barringtonia acutangula*, reported about anticancer activity. Recently endophytic fungi from healthy mangrove leaves of Thailand, reported to have antifungal activity, it has been reported that endophytic directly suppress pathogens by producing antibiotic or lytic enzymes. Further endophytes isolated from trophical plants were reported as the largest reservoir of bioactive secondary metabolites. *Pythium ultimum*, compound was purified and named as xyolide and its structure was elucidated by NMR method (Baraban et al., 2013).

Form the reports published novel antimicrobial metabolites from endophytes is an important alternative to the drug resistance human pathogens. Endophytic antimicrobial activity reported from the endophytes from *Garcinia mangostana* (Radji et al., 2013). Further antibacterial terpene was isolated from the endophytic fungi from *Allamanda cathartica*.

Immunosuppressive activity reported from the endophytes *Colletotrichum dematium* isolated from *Pteromischum* sp. growing in tropical forest of Costa Rica produced novel antimycotic peptide collutelin A. Collutelin A exhibited strong immnosuppressive activity as it inhibited CD4 (+) T cell activation of Interleukin 2 production (Ren et al., 2008).

Anticancer properties of the microbial products camptothecin and two analogues (9-Methoxycamptothecin and 10-Hydroxycamptothecin were reported from the endophytic fungi *Fusarium solani* isolated from *Camptotheca acuminata* (Kusari et al., 2009). Methodology were developed for isolation, mixture separation and production of camptothecin and its analogues from novel endophytic fungal sources associated with *Nothapodytes foetida* and other plant species (Puri et al., 2006; Amna et al., 2006; Shweta et al., 2010). *Acremonium* sp, an endophyte in European yew
(Taxus baccata) reported production of peptide with antifungal-anticancer agents known as the leucinostatins (Strobel et al., 1999). Similarly, phomoxanthones A and B, two novel xanthone dimers, were isolated from the endophytic fungus Phomopsis sp. BCC 1323. These compounds exhibited significant in vitro antimalarial, antitubercular and anticancer activities (Isaka et al., 2001). A novel anticancer agent Ergoflavin reported from an endophytic fungus growing on the leaves of an Indian medicinal plant Mimusops elengi (Sapotaceae) (Deshmukh et al., 2011). Similarly Secalonic acid D, a mycotoxin (ergochrome class), is known to have potent anticancer activities was isolated from the mangrove endophytic fungus and observed high cytotoxicity on HL60 and K562 cells by inducing leukemia cell apoptosis (Zhang et al., 2009).

Endophytes associated with leaves of tropical plants are relatively untrapped source of novel compounds. Endophytic fungal extracts from the regions of Panama reported toxic against human breast cancer cell line (MCF-7) and against causative agents of malaria, leishmaniasis. The research suggested that using host taxonomy and forest type, for selecting endophytes would increase the efficiency and efficacy of discovering bioactive metabolites for particular pharmaceutical targets.

V. album

V. album is a hemiparasitic plant, grows on different host tree, It has been reported that V. album was a parasitic plant belongs to Loranthaceae, growing of many deciduous lemon trees it was reported to have various medical uses, its phytochemical screening showed the presence of alkaloids, tannins, flavanoids, phytosterols and lectins, ethanolic extract of V. album inhibited the growth of pathogenic bacteria reporting its antibacterial activity (Oguntoye et al., 2008). A similar type of phytochemical constituents of V. album was reported and found to contain various phytochemicals such as glycosides, alkaloids, viscotoxins, phenylpropanoids, tannins, lignins and sugars (Jurin, 1993). Similar types phytochemical constituents was reported from V. album growing five different plants Psidium guajava, Cola nitida, Citrus, Persea americana and Theobroma cacao (Taiga, 2013). Antitumorous and immunomodulatory activity was observed from the V. album (Jurin, 1993).
Extracts of *V. album* used as complementary cancer therapies in Europe, lectins and Viscotoxin are the active principle component involving in biological activity and reported to have cytotoxic activity in tumor cells (Vicas *et al.*, 2008). Antihypertensive effect was reported in aqueous extract of *V. album* leaves, in the *in vitro* studies using albino Wistar rats (Ofem *et al.*, 2007).

 Phenolic compounds are one the active ingredient of *V. album*, amount of phenolic acid present in *V. album* growing on various hosts was analyzed using HPLC. Comparative chromatography revealed qualitative differences in the investigated compounds between various plant materials; thus concluded that host influences the accumulation of bioactive compounds in the *V. album* (Luczkiewicz *et al.*, 2001). Similar report of phenol content in *V. album* will be influenced by the host, methanolic extract of *V. album* growing on Cocoa and cashew trees in Nigeria, reported host dependency in total phenol content and antioxidant capacity (Oluwaseun *et al.*, 2008). A further polyphenolic compound from *V. album* reported to have excellent antioxidant and antimutagenic potencies, polyphenolic extract protected human lymphocytes against trichloroethylene (TCE) induced oxidation and genotoxic damage (Alpsoy *et al.*, 2010).

 The antioxidant capacity of *V. album* proved to be dependent on the host tree on which it grows, significant decrease in phenolic derivatives (pheholic acids and flavonoids) was reported from *V. album* growing on different host, but no significant difference was noticed between the antioxidant content and activity of different plant parts of *V. album* (stems versus leaves), phenolic compounds reported to have antioxidant activity. Difference in antioxidant activity between leaves and stems of mistletoes obtained from different host is due to variation in environmental factors, which affect the accumulation of antioxidant components. Research concluded that the host tree of *V. album* plays an important parameter in the assessment of the phytopharmaceutical evaluation (Vicas *et al.*, 2008).

Antimicrobial activity of organic solvent extract of *V. album*, showed excellent antimicrobial activity against pathogenic bacteria, it is concluded that the leaves and twigs of *V. album* are regarded as good natural antibiotics with a considerable degree
of antimicrobial activity (Hussain et al., 2007). It has been reported that \textit{V. album} growing on different host will influence on its antitumor properties (Turker et al., 2012).

The cytotoxic effect of the \textit{V. album} extract growing on various hosts was reported on various cancer cell lines, alkaloids, viscotoxin and lectins were probably responsible for their cytotoxicity, it has been concluded that cytotoxicity of the extract depends on the host tree and extraction methods employed. Aqueous extract of \textit{V. album} preparations has been used as complementary medicine, clinical studies have reported that \textit{V. album} preparations improved the quality of life of cancer patients, biological active components like lectins, viscotoxins, polyphenols and polysaccharides influence the immunomodulatory and anti tumor effects (Elluru et al., 2007). Similarly \textit{V. album} extract reported to have therapeutic effects on gynecological and breast cancer, is has been reported that \textit{V. album} extract showed an increase in survival in mice and compound have a string effects on cancer cells (Kienle et al., 2009).

Extract of \textit{V. album} growing on \textit{Mallus domestic}, reported to modulate the activity of phase II detoxifying enzymes using the glutathione S-transferase (GST) and quinone oxidoreductase (QR) assays and to inhibition of adherent epithelial human ovarian tumor cells A2780 proliferation using the MTT assay, and also reported that extract is the good antioxidant source (Vicas et al., 2011).

**Lectin**

Lectin are glycoproteins of non immune origin, term hemagglutinin is applicable because of the ability to agglutinate erythrocytes. This property was first reported in Castor bean (Stillmark, 1986). William et al. (1995) coined the term lectin, it has been reported that it has least one catalytic domain that bind reversibly to specific monosaccharides. Lectins have the ability to agglutinate the cells and react with glycoconjugates present on their cell surface. Lectins are carbohydrates binding proteins that specifically recognize diverse sugar structures and induce a variety of
biological processes. Lectins bind reversibly to the carbohydrates and do not induce any change in the bound carbohydrates (Peumans and Van Damme, 1995).

The first sugar specificity of lectin was reported in Concanavalin A and crystallized. Most remarkable discoveries that brought lectin to lime light is phytohemagglutinin from Phaseolus vulgaris reported to possess mitogenic activity, it stimulates lymphocytes to undergo mitosis, this research provided the proof that mitogenic stimulation is due to binding of lectin to sugars on the surface of lymphocytes, later mitogenic lectins became a tools for signal transmission in to the cells. Similarly, wheat germ agglutinin reported to agglutinate malignant cells, this provided the evidence that cell surface sugars are associated with the development of cancer. Numerous lectins have been reported from various source plant lectins from soya bean, green peas, wheat germ and animal lectin.

Presence of lectin in the sample by periodic acid Schiff stain on a nondenaturing polyacrylamide gel was reported by (Kinchel et al., 1990). Lectin was isolated and partially purified using affinity chromatography, from seeds of Cissus poplunea, hemagglutination assay showed nonspecific agglutination of human blood types, various carbohydrate specificity were studied (Awoyinka and Dadaoo, 2011). The adherence of bacteria to host cells is mediated by lectin-like adhesions on the bacterial cell surface, which recognize carbohydrate receptors (Ofek and Sharon, 1988). Similarly retrovirus such as human immunodeficiency virus (HIV) and other enveloped virus are reported to cover with glycoproteins.

Antibacterial activity of lectin was first reported from wheat flour. Similarly Bothrops leucurus letcin, exhibited antibacterial effect against the human pathogenic Gram positive bacteria S. aureus, Enterococcus faecalis and B. subtilis (Nunes et al., 2011). Further Archidendron jiringa seed lectin reported inhibitory activity against B. subtilis and S. aureus (Charungchitrak et al., 2011). Lectins from serum, plasma, skin, mucus and egg of fishes studied (Dong et al., 2004; Tasumi et al., 2004). Lectin from Eugenia uniflora seeds showed a potential antibacterial effect, reported to be high potential for therapeutic applications (Oliveira et al., 2008). Antifungal activity by
inhibition of germination of spores and hyphal growth was reported by mannose/glucose specific lectin from Capsicum annum (Kuku et al., 2009).

The antiviral activity of a number of lectins that bind high-mannose carbohydrates has been reported in the past. Such lectins include jacalin (O'Keefe et al., 1997), concanavalin A (Hansen et al., 1989), Urtica dioica agglutinin (Balzarini et al., 1992), Myrianthus holstii lectin (Charan et al., 2000) and Narcissus pseudonarcissus lectin. However, lectins derived from marine organisms, a rich source of natural antiviral products (Tziveleka et al., 2003), such as CV-N (Boyd et al., 1997), SVN (Bokesch et al., 2003), MVL (Bewley et al., 2004) and GRFT (Mori et al., 2005), exhibit the highest activity among the lectins that have been investigated so far (Ziółkowska and Wlodawer, 2007).

Some lectins found in algae, such as cyanovirin-N (CV-N) (Boyd et al., 1997; Esser et al., 1999; Barrientos et al., 2003); scytovirin (SVN) (Bokesch et al., 2003), Microcystis viridis lectin (MVL) (Bewley et al., 2004) and griffithsin (GRFT) (Mori et al., 2005) exhibit significant activity against human immunodeficiency virus (HIV) and other enveloped viruses, which makes them particularly promising targets for the development as novel antiviral drugs. The antiviral activity of lectin is mediated by the interaction of lectin with mannose containing oligosaccharide on the surface of viral envelope this reported to disturb interactions between the proteins of viral envelop and the cells of the host (Balzarini, 2006).

The carbohydrate binding profile of the red algal lectin KAA-2 from Kappaphycus alvarezii was reported by (Sato and Morimoto, 2011). They tested the anti-influenza virus activity of KAA-2 against various strains, including the recent pandemic H1N1-2009 influenza virus. KAA-2 inhibited infection of various influenza strains with EC50 of low nanomolar levels. Immunofluorescence microscopy using an anti-influenza antibody demonstrated that the antiviral activity of KAA-2 was exerted by interference with virus entry into host cells. This mechanism was further confirmed by evidence of direct binding of KAA-2 to a viral envelope protein, hemagglutinin (HA), using an ELISA assay. These results indicate that this lectin could be a useful antiviral agent.
Lectin mediated in specific drug delivery was reported by Woodley and Naisbett in 1988. Drug delivery by lectin had increased the efficacy of treatment and limiting the side effect. Similarly lectin based drug delivery has been targeted on glycosylated receptors at the cell membrane; this induces active receptor mediated endocytosis, and improve cytoinvasion of prodrugs. Lectin antitumor effect has been well documented, predominantly galactoside and galNAc specific lectins has been employed as potential drugs for treatment of cancer (Ernst et al., 2003). Lectin from Arisanema tortusum reported to have anticancer activity on human cancer cell lines HT29, SiHa and OVCAR-5 (Dhun et al., 2005). Similarly, two N-acetyl-D-lactoseamine specific lectins was isolated and reported to have an antiproliferative effect against human cancer cell lines (Kaur et al., 2005).

Legume lectins are one of the extensively studied plant lectin for several decades. Lectin anti tumor potential depends on binding to glycoconjugate on the cancer cell surface. Concanavalin A, a legume lectin with mannose/glucose binding specificity, reported to induce apoptosis in human melanomas cells in a caspase dependent pathway and triggering a mitochondrial mediated apoptosis. Further, Flammulina velutipes hemagglutinin-inhibited the proliferation of leukemia L1210 cells. Haliclona crater lectin displayed a cytotoxic effect on HeLa and FemX cells (Pajic et al., 2002). Dark red kidney bean hemagglutinin exerted an antiproliferative activity toward leukemia L1210 cells. Small glossy black soybean (Glycine max) lectin impeded proliferation of breast cancer MCF7 cells and hepatoma HepG2 cells.

*In vitro* antitumor activity of Lusca domestica lectin reported the inhibition of proliferation of human breast cancer (MCF-7), apoptosis as induced through caspase-3 and mitochondria mediated pathway, DNA fragmentation was observed as one of the characteristic features of apoptosis (Cao et al., 2010).
Review of Literature

Lectin from V. album

Toxic protein was isolated from the V. album, purified through affinity chromatography, molecular weight of isolated protein was found to be 60 k Da, after digesting with 2-mercaptoethanol two bands were observed having the molecular weight of 29 and 34 k Da, this confirms that protein is made up to two chains, isolated protein was capable of inhibiting protein synthesis, protein was identified as viscumin (Olsnes et al., 1982). Mistletoe lectins are the conjugate of A-chain and B-chain, its cytotoxicity effect is by the inhibition of protein synthesis, it has been reported that immunopotentiating properties of mistletoe lectins and their chains are responsible for mediating the therapeutic effects (Franz et al., 1981).

Chitin binding lectin was isolated from the V. album, this lectin was completely differs from classical galactose/N-acetyl-galactosamine binding mistletoe lectins, but the cytotoxic properties is less than the other mistletoe lectin (Peumans and Van Damme, 1995). Lectin from Korean mistletoe an subspecies of V. album growing in Quercys mongolica, reported to be to D-galactose and N-acetyl-D-galactosamine, specific MALDI analysis showed the average mass of 62.7 kDa, made up to two subunits having a molecular weight of 30.6 kDa and 32 kDa.

Immunoactivating effects of mistletoe lectin have been observed three lectins from V. album (VAA-I, VAA-II, VAA-III) showed the secretion of cytokines tumor necrosis factor, interleukins at small concentration this shows that mistletoe letcins may have important value in cancer treatment. Similar immunomodulatory activities of V. album agglutinin-I (VAA-I) was found to be involved in enhanced secretions of proinflammatory cytokines, stimulates the natural killers it has been reported that VAA-I balances between cell growth and programmed death (Hajto 1990; Lavastre et al., 2000). Similarly immunomodulatory activities of lectin from V. album coloratum reported the proliferation of murine splenocytes and act to balance of Th1/Th2 cellular response (Lyu and Won, 2006).

The antitumor action of V. album coloratum agglutinin (VCA), from Korean mistletoe, reported that VCA induces apoptosis by the rapid changes in mitochondrial
transition permeability, caspase-3 activity, polymerase degradation, C-JunNH2-terminal kinase activation and DNA fragmentation in Hep3B cells (Kim et al., 2004). Further the treatment of breast cancer by VAA-I studies revealed that galactose containing glycoprotein in breast cancer cells, VAA-I interact with cell surface carbohydrates and exert a therapeutic effect in turn modulated the immune cells (Fritz et al., 2004). Protein sequence and the X-ray studies of mistletoe lectin I (ML-I), reported that A - chain has 254 amino acids, B-chain has 264 amino acids, linked through disulfides bridges, structural identification reveals that ML-I is a member of the ribosome inactivating protein, the X-ray structure clearly represents its toxic effects and immunomodulating activities and reported for treatment for cancer patients (Voelter et al., 1998).

The antitumor activity of V. album coloratum agglutinin on human colon cancer cell lines was observed in a time and dose dependent manner, apoptosis was induced in cancer cells through the activation of caspase-2,-3,-8 and -9 and inhibition of apoptosis protein (Lee et al., 2012). Lectin from Korean mistletoe was isolated and reported the molecular weight of 64 kDa, made of two subunits linked through disulfide bonds; carbohydrate binding specificity to Gal and GalNAc, results reported that Korean mistletoe having anticancer properties. V. album agglutinin-I (VAA-I) induced apoptosis in leukemia PLB-985 through caspases that are involved in cytoskeletal protein degradation, it has also been reported that VAA-I posses potential therapeutic properties along with immunomodulatory activities (Lavastre et al., 2000). Because of enormous advantage of V. album agglutinin (VAA), recombinant mistletoe lectin expressed in E.coli, recombinant lectin was used in combination with ionizing radiation or alone, it has been reported that apoptosis was induced in tumor cells by the activation of caspase-3 cells (Hostanska et al., 2003).

Recently the recombinant VAA-I was expressed with Pichia pastoris system, cytotoxic effect of recombinant VAA-I on human hepatocellular carcinoma cells (SMMC-7721) a series of genes mitogen activates protein kinase are expressed differently and induced apoptosis in cancer cells via the activation of PI3-kinase pathway (Yang et al., 2012).