Introduction

In India, cancer has become second most common disease with high alarming mortality rate of 6,35,000 people in the year 2008 and representing about 8% of all estimated global cancer deaths (Dikshit et al., 2012). This is owing to the poor availability of prevention, diagnosis and treatment of this deadly disease. According to Indian census data in 1991, about 6,09,000 cancer cases have been observed. This number had drastically increased to 8,06,000 by the end of last century (Rao and Ganesh, 1998). It is believed that in near future the number of cancer patients will increase. The most affected states in India are Jammu & Kashmir, Himachal Pradesh, Delhi, Uttarakhand, Rajasthan, Maharashtra, Jharkhand, West Bengal, Andhra Pradesh, Kerala, Tripura and Manipur. All type of cancers have been reported in Indian population including skin, lung, breast, rectum, stomach, prostate, liver, cervix, esophagus, bladder, blood, mouth etc. Among them, Cervical cancer is found to be the second most common form of malignancy in female population of Himachal Pradesh, Haryana, Rajasthan, Goa, Tamil Nadu and West Bengal. The prevalence of cancer in India is affecting the economy of the country by spending quantum of wealth on buying medicine, hospitalization, pathological tests, medical practitioner consultancy, travel, lodging and so on. The total number of cancer patients in 2004 was 8,19,354 with a total loss of $215.16 million, whereas in the year of 2009 it became 9,62,832 people and loss of $270.06 million. The total cancer patients in the year 2010 were 9,79,786 with total economic loss of 274.10 million US $ (Ali et al., 2011).

International agency for research on cancer has estimated that 14.1 million new cancer cases, and 8.2 million of deaths have been occurred globally in the year 2012. Nearly ten million new cases have been diagnosed annually around the world and out of these about half of the cases are only from the developing countries. It is predicted that by the end of 2020, over 10 million people would die globally because
of cancer with 70% mortality is only from the developing countries (Murray and Lopez, 1996).

1.1. Cancer

Cancer is a group of disease which cause by multiple alterations in gene expression resulting in deregulation of normal cell functions, imbalance of cell proliferations and cell death. It leads to the development of abnormal cell population which can metastasize to other organs and transform the normal cells into cancer cells. Cancer may occur by various carcinogenic factors such as exogenous chemical, radiations, biological carcinogens, endogenous processes and genetic defects. Mechanism of carcinogenesis differs with the type of carcinogens and type of organs or cells being transformed. But all carcinogens are causing DNA damage, ultimately produce the cancerous cells (Schulz, 2005; Ruddon, 2007).

1.2. Hallmark of cancer

During multistep progression of cancer, it acquire specific biological characters called hallmarks of cancers, they rationalize neoplastic disease. Hallmark of cancer cells comprise six biological capabilities that include 1) sustaining proliferative signaling, 2) evading growth suppressors, 3) resisting cell death, 4) enabling replicative immortality, 5) inducing angiogenesis 6) activating invasion and metastasis (Fig. 1). Recently two more characters such as reprogramming of energy metabolism and evading immune system had been added to cancer hallmark. In addition to cancer cells, tumors exhibit another dimension of complexity. They contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the “tumor microenvironment”. Recognition of the widespread applicability of these concepts; will increasingly affect
the development of new means to treat human cancer (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011).

Fig.1. Schematic representation of Hallmark of cancer cells.

1.3. Programmed Cell Death

Programmed cell death (PCD) is also known as apoptotic cell death. The term apoptosis was first coined by Kerr in year of 1972. Apoptosis is genetically programmed mechanism which allows the cell to commit suicide and rid of damaged or infected cells in human body. Apoptosis involves energy depended cascade molecular events like protein cleavage, protein cross-linking and DNA break down. All together causes changes in morphology of cells including cell-shrinkage, pyknosis, membrane blebbing, karyorrhexis finally cells are getting fragment into apoptotic bodies through budding. Apoptotic bodies appear as oval or round shape, containing tightly packed cytoplasm and other cellular organelles; at last these bodies are phagocytosed by macrophages. Macrophages that engulf and digest apoptotic cells are called “tingible body macrophages” and are frequently found within the reactive
germinal centers of lymphoid follicles or occasionally within the thymic cortex. The tingible bodies are the bits of nuclear debris from the apoptotic cells. There is essentially neither inflammatory reaction associated with the process of apoptosis nor with the removal of apoptotic cells. Because apoptotic cells do not release their cellular constituents into the surrounding interstitial tissue, they are quickly phagocytosed by surrounding cells thus; likely preventing secondary necrosis and the engulfing cells do not produce anti-inflammatory cytokines.

There are two main classification of apoptotic pathways were identified namely intrinsic and extrinsic pathways. The intrinsic signaling pathway is non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell and mitochondrial-initiated events. Whereas extrinsic pathway is mediated by receptors of Tumor Necrosis Factor superfamily which includes Apo2L/DR4, Apo2L/DR5, FasL/FasR and TNF-α/TNFR1 receptors. Defect in apoptosis process leads to complicated disease like cancer, autoimmune disorders, neurodegenerative diseases and ischemic damage (Elmore, 2007; Portt, 2011).

1.4. TRAIL Induced Extrisic Apoptosis

Extrisic apoptosis can be triggered by activation of cell surface death receptors DR4/DR5, FasR and TNFR through binding upon their ligands such as TRAIL, FasL and TNF (Fig. 2). Among them TRAIL mediated apoptosis acts only on cancer cell and it have no toxicity towards normal cells (Ashkenazi, 1999; Ashkenazi, 2008), whereas Fas and TNF showed adverse effect in normal cells.

Tumor Necrosis Factor-α (TNF α) - Related Apoptosis Inducing Ligand (TRAIL/Apo 2L) belongs to TNF cytokine family (Wiley et al., 1995). It selectively induces apoptosis on cancer cells through binding with two death receptors (DR)
namely TRAIL-R1 (DR4) and TRAIL-R2 (DR5) (Pan et al., 1997b; Walczak et al., 1997) (Fig. 2). This binding cause trimerization of TRAIL-Rs and activate its intracellular death domain (DD) (Bodmer et al., 2000a). Followed by adapter molecule FAAD (Fas-associated Death Domain) translocated to DRs, via death effector domain (DED) interaction. FAAD recruits pro-caspase 8/10, resulted in formation of multi-protein death inducing signaling complex (DISC) where pro-caspase 8/10 is auto-catalytically activated (Kischkel et al., 2000; Sprick et al., 2000).

![Schematic diagram of the human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated extrinsic apoptosis pathway.](image)

Cleaved caspase 8/10 subsequently activate effector caspase 3 and apoptosis takes place, this path is known as type 1 extrinsic apoptosis. In some circumstance extrinsic apoptosis is linked with mitochondrial depended intrinsic apoptosis and are
called type 2 extrinsic apoptosis. In that case, caspase 8/10 converts Bid into tBid. tBid is translocated to mitochondria where activate Bax and Bak, consequently release Cytochrome C. Cytochrome C is associated with dATP, Apoptotic protease activating factor 1 (Apaf-1) and Caspase 9. All together forms a protein complex called apoptosome, where pro-caspase 9 turn into activated caspase 9 and in turn activates downstream effector caspases 3, 7 and 9. Moreover, second mitochondria derived activator Smac/DIABLO is also released from mitochondria during apoptosis. It counteracts the function of X-linked inhibitor of apoptosis protein (XIAP) to enhance the apopototic cell death (Ozoren et al., 2000; Ozoren and Deriy, 2002).

1.5. TRAIL and TRAIL Receptors

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is also known as Apo2 ligand (Apo2L). It is a type II membrane protein and belongs to cytokine of TNF superfamily (Wiley et al., 1995; Pitti et al., 1996). TRAIL is expressed on surface of the immune cells include interferon IFN-stimulated monocytes, natural killer (NK) cells, dendritic cells, fibroblasts, T-cells and interleukin-2 (IL-2) stimulated NK cells (Takeda et al., 2001; Almasan and Ashkenazi, 2003. Bouralexis et al., 2003; Falschlehner et al., 2009). The TRAIL gene is located on chromosome 3 at position 3926. TRAIL protein consists of 291 amino acid having C-terminal extracellular domain which shows homology to other TNF family members (Fig. 3). This extracellular domain is cleaved from surface of immune cells by proteolytic action of cysteine proteases generating soluble form of TRAIL ligand (Wajant et al., 2001).
TRAIL consists of two antiparallel β-pleated sheets that form a β-sandwich and interacts with the adjacent subunits in a head-to-tail fashion forming a bell-shaped homotrimer (Cha et al., 1999; Hymowitz et al., 1999; Mongkolsapaya et al., 1999a). Each TRAIL monomer contains one cysteine at position 230. The side chains of each Cys-230 form a unique zinc-binding site buried at the core of the trimer. The zinc ion is essential for structure, stability and biological activity of TRAIL (Bodmer et al., 2000b; Hymowitz et al., 2000) (Fig. 3.). TRAIL forms a homotrimer which binds one copy of the receptor in each of the identical clefts between the TRAIL subunits.

TRAIL interacts with five distinct receptors, namely DR4 (Death Receptor 4/TRAIL-R1) (Pan et al., 1997b). DR5 (Death Receptor 5/TRAIL-R2/Killer) (Walczak et al., 1997; Chaudhary et al., 1997; Schneider et al., 1997), DcR1 (Decoy Receptor 1/ TRAIL-R3/TRID/LIT) (Degli-Esposti et al., 1997a; Pan et al., 1997a), DcR2 (Decoy Receptor 2/TRAIL-R4/TRUNDD) (Degli-Esposti et al., 1997b; Marsters et al., 1997), and a soluble receptor called osteoprotegerin (OPG) (Fig. 4).
Among them, only DR4 and DR5 are having agonistic which contain an intracellular Death Domain (DD) in cytoplasmic region can recruit pro-Caspase-8/10 consequently activate apoptosis process. Approximately 80 amino acid of intracellular death domain is essential for transmitting the apoptotic signal (Nagata, 1997; Ashkenazi and Dixit, 1998) upon binding of TRAIL with DR4/5. However a clear distinction in the roles of DR4 and DR5 has yet to be established. DcR1 and Dc R2 are having close homology with the extracellular domains of the agonistic receptors DR4 and DR5. But DcR2 has a truncated, nonfunctional cytoplasmic death domain, whereas DcR1 doesn’t have DD, it exists on the membrane as a glycophospholipid - anchored protein lacking of a cytosolic region. Hence, it couldn’t transmit the apoptosis signal into cells and this binding cannot induce the apoptotic machinery. DcRs are competing with DR4 and DR5 for binding to TRAIL. Over expression of DcRs block apoptosis in cancer cells. TRAIL also binds with OPG receptor with low affinity. It also fails to induce apoptosis in cells.

![Fig. 4. TRAIL interacting receptors. The extracellular TRAIL ligand binding with death receptors, DR5 and DR4, contain a DD in their intracellular region, which is essential for apoptosis signaling. Decoy receptors DcR1 and DcR2 are devoid of intracellular dead domain. OPG, a soluble receptor, is capable of binding to TRAIL with low affinity.](image-url)
1.6. The potential of TRAIL as a cancer therapeutic agent.

The soluble recombinant TRAIL is promising agent for cancer therapy for a number of reasons. Targeting cancer cell and leaving non transformed cell is the goal of cancer therapy. There are few agents that are truly cancer cell-specific in terms of efficacy or cell death induction. TRAIL is a rare example of such molecules that kill many transformed cells but not the normal cells (Ashkenazi and Dixit, 1998). Importantly, administration of soluble recombinant TRAIL in experimental animals, including mice and primates, induces significant tumor regression without systemic toxicity (Ashkenazi et al., 1999; Walczak et al., 1999). Pre-clinical studies have confirmed the potential of soluble rhTRAIL which targeting either DR4 or DR5 and selectively induce the apoptosis in a variety of cell lines. This led to the progression of TRAIL-based targeted therapies to clinical trials either as single agents or in conjunction with a range of conventional cancer therapeutics whose effects have been shown to be enhanced upon combination (Jin et al., 2008; Luster et al., 2009; Marini et al., 2009).

Preclinical studies have found that rhTRAIL induce apoptosis in a number of cancer cell lines and have broad-spectrum activity against human malignancies (Walczak et al., 1999). Allometric scaling provided estimates of Apo2L/TRAIL kinetics in humans, suggesting that on a milligram per kilogram basis, doses significantly lower than those used in xenograft studies could be effective in humans (Kelley et al., 2001).

A number of phase 1 clinical studies had revealed that rhTRAIL to be safe and well tolerated at the doses tested and no hepatotoxicity in patients with advanced solid
tumours (Herbst et al., 2006; Ling et al., 2006; Pan et al., 2007; Herbst et al., 2010). However, TRAIL having certain limitations in response with cancer cells.

1.7. Human papillomaviruses (HPV)

Human papillomaviruses are small non enveloped, 55nm diameter icosahedral capsid, containing double-stranded circular DNA of ~8,000bp, which can infect epithelial cells. Currently, about 200 different HPV types are recognized, among them 120 HPV types are fully sequenced. HPVs have been traditionally referred with their ‘‘types’’, L1 nucleotide sequence of HPV is at least 10% dissimilar from other HPV types. The HPV types have also been grouped into mucosal or cutaneous types, based on their tropism for specific epithelial sites (Munger et al., 2004; Rautava and Syrjanen, 2012).

HPVs are further divided into two types called low risk and high risk groups, according to their capability of inducing lesions and progress to malignancy. Most of the HPVs belongs to low risk group such as types 6 and 11 etc. They are associated with genital warts that do not undergo malignant progression even if left untreated. While the high risk HPV types cause intraepithelial lesions that can progress into invasive carcinomas. High risk HPV types are associated with 99.7% of cervical cancers. High-risk HPVs types include 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Among them HPV 16 and 18 are most virulent types. (Beaudenon and Huibregtse, 2008).

1.8. HPV 16 and Cervical cancer.

Human papillomavirus are etiological agents in nearly all cases (99.7%) of cervical cancer. Cervical cancer is the fifth most common cancer in human and
second most common cancer in women. Every year, approximately 510,000 new cases are diagnosed with cervical cancer and approximately 288,000 women die worldwide, majority (~80%) of these cases and deaths were reported from developing countries. India has ~365.71 million cases of cervical cancer, 1,32,000 newly diagnosed and annual death is 74,000 women. It’s nearly 1/3rd of the global cervical cancer deaths. HPV serotype 16 causes nearly 76.7% of cervical cancer in India (Kaarthigeyan, 2012).

![Fig. 5. Schematic representation of the HPV-16 double-stranded circular DNA genome.](image)

The HPV16 virion is 55 nm in diameter size and contains T=7 icosahedral capsid composed of double standard DNA (Fig.5). Genomic DNA is divided into early region (E), a late region (L) and a non-coding long control region (LCR). The E region encodes seven non-structural proteins: E1, E2, E4, E5, E6, E6 and E7, while the L region encodes two structural proteins: L1 and L2, which code for the major and minor capsid proteins, respectively (Fig. 5). Early genes are responsible for
modulating epithelial cell function which favor or promote the viral DNA replication and segregation, and inhibit viral clearance by the immune system. Increased expression of HPV early proteins, including the E6 and E7 oncoproteins plays an important role in cellular transformation and carcinogenesis. (Yuan et al., 2012).

1.9. Modulation of Apoptosis by HPV Oncoproteins E6 and E7

E6 is a small protein, consisting of 151 amino acids and presenting two atypical zinc fingers with motifs that contain two cysteines (Cys-X-X-Cys). It is known to suppress p53, E6 from high risk types of HPV interferes with process of p53 and E6-associated protein ligase (E6AP), causing ubiquitinylation and the subsequent degradation of p53. E6 has significance in both the viral life cycle and in carcinogenesis. E6 interact with other apoptosis such as FADD, caspase-8, Bak, c-Myc and TNF R1. E6 can bind to the death effector domains (DEDs) of FADD and procaspase-8 and accelerate their degradation. The resulting lower amounts of FADD and procaspase 8 in E6-expressing cells hinders formation of the apoptotic Death Inducing Signaling Complex (DISC), thereby compromising the ability of TRAIL, TNF and FasL (Filippova et al., 2004; Filippova et al., 2005; Garnett et al., 2006; Filippova et al., 2009) (Fig. 6). Over expression of E6 in SiHa cells was shown to bind with pro-caspase 8, that affect its stability in opposite directions, leading to instability and degradation of pro-caspase 8. During carcinogenesis, the expression of E6 is usually increased, result in that, cells do not undergo apoptosis (Yuan et al., 2012).

E7 oncoprotein is necessary for viral pathogenesis and cellular transformation. E7 is small acidic polypeptide composed of approximately 100 amino acids (McLaughlin-Drubin et al., 2009) that shares functional similarities with other viral
oncoproteins. The N-terminus of E7 contains two conserved regions (CRs), CR1 and CR2. It inhibits TNF-α-mediated apoptosis in normal human fibroblasts by upregulating the expression of the inhibitor of apoptosis (IAP) protein, c-IAP2, and suppressing caspase 8 activation. So cancer cells are able to escape from apoptosis function (Jiang et al., 2014).

![Fig. 6. Modulation of apoptosis by HPV early proteins.](image)

1.10. Mechanism of TRAIL resistance

The best response was observed in patients from phase I and II trials of rhTRAIL. But clinical observation seems to reveal that vast majority of human cancers are resistant to apoptosis induction by TRAIL. Some cancer cells that were originally sensitive to TRAIL-induced apoptosis can become resistant after repeated exposure (acquired resistance) to TRAIL (Thorburn et al., 2008). Unraveling the
molecular mechanisms in TRAIL resistance provides new approaches which target the resistance mechanisms to overcome the cancer resistance to TRAIL-based cancer therapies.

Resistance to TRAIL can occur at different points in the signaling pathways of TRAIL induced apoptosis. Dysfunctions or low expression of the death receptors DR4 and DR5 can lead to resistance. The adaptor protein Fas-associated death domain (FADD) and caspase 8 are essential for assembly of the death-inducing signaling complex (DISC), defects in either of these molecules can lead to TRAIL resistance. Overexpression of cellular FADD-like interleukin-1b-converting enzyme inhibitory protein (cFLIP) correlates with TRAIL resistance in several types of cancer. Overexpression of Bcl-2 or Bcl-XL or loss of Bax or Bak function, high expression of inhibitor of apoptosis proteins, and reduced release of Smac/Diablo from the mitochondria to the cytosol have all been reported to result in TRAIL resistance in mitochondria dependent type II cancer cells. Activation of different subunits of mitogen-activated protein kinases or nuclear factor-kappa B can lead to development of either TRAIL resistance (Zhang et al., 2005).

DR4 and DR5 initiates the TRAIL-induced signalling pathway, therefore these receptors can be the first point of resistance. Deaths receptors must be expressed on the cell surface in sufficient amounts and must be functional in order to successfully commence signalling. Studies have shown that a lack of expression of DR4 in ovarian cancer cells can be correlated with the resistance to TRAIL-induced apoptosis. This lack of expression was caused by epigenetic silencing (Horak et al., 2005). Mutations in either DR4 or DR5 have been observed which result in loss-of-function and consequent resistance to TRAIL-mediated cell death.
(Fisher et al., 2001; Bin et al., 2007). TRAIL could to bind with non apoptosis inducing decoy receptors. It can reduce the amount of ligand available to bind to the death inducing receptors, thereby having reduced efficacy. While decoy receptors are present on normal cells, evading them will not cause the normal cells to succumb to TRAIL mediated apoptosis. If the cancer decoy receptors are over expressed that will results in the reduction of the efficacy of TRAIL, and resistance occurs in certain cancer cell lines (Sanlioglu et al., 2005; Koksal et al., 2008; Chamuleau et al., 2011).

In many cases resistance to TRAIL-induced apoptosis has been found to be a result of high levels of cellular FLICE inhibitory protein (c-FLIP) expression (Tschopp et al., 1998). c-FLIP has a short and a long isoform, as a result of two different splice variants. The short form, c-FLIPS, consists only of two DEDs and the long form, c-FLIPL (also called I-FLICE, for inhibitor of FLICE) consists of two DEDs and a caspase-like domain, and closely resembles caspase-8, where the major difference is that it contains an inactive enzymatic site. These two isoforms differ in that if c-FLIPS is incorporated into the DISC, it can competitively inhibit pro-caspase-8 recruitment into FAAD.

1.11. **Combination of rhTRAIL with naturally occurring compounds.**

Unfortunately, cancers eventually become resistant to chemotherapy. The current challenge is to overcome TRAIL resistance and make the cancer cells sensitize to TRAIL. Recent studies have shown that chemotherapeutic agents at non-toxic doses can enhance TRAIL-induced apoptosis in cancer cells, although the molecular mechanisms by which TRAIL synergizes with chemotherapy remain controversial. Cancer therapeutic agent like cisplatin and etoposide upregulate DR4 and DR5 mRNA (Lacour et al., 2001) inhibits c-FLIP expression (Song et al., 2003).
Combining TRAIL with these other types of therapeutic agents, especially naturally occurring polyphenolic can restore tumor cell sensitivity to rhTRAIL allows the dual activation of both the intrinsic and extrinsic apoptotic pathway which has been shown to amplify their effects (Jacquemin et al., 2010).

1.12. **Mangrove plants and its therapeutic potential**

Mangroves are halophytic woody plants growing in brackish wetlands in the tropical and sub-tropical inter-tidal coastal zones and river deltas where other plants cannot grow (Kathiresan, 2000; Arumugam and Panneerselvam, 2012; Simlai and Roy, 2013). There are about 39.3 million acres of mangrove forests in the warm coastlines of tropical oceans all over the world and giving protection from natural calamities such as cyclone, Tsunami, erosion etc. It provides food and shelter for a large number of commercially valuable fin- and shell-fishes and it also plays a vital role in maintenance of food chain in marine ecosystem. Mangrove habitats are ecologically important as they function as natural nutrient filters and recyclers, aid in floodwater mitigation and protect coastal areas from seawater intrusion.

Mangroves plants possess very good medicinal properties and this has been established by research over years. The screening of anticancer compounds from mangrove plants helps to identify the potential candidates. Mangroves have been traditionally used for medicinal purposes in different parts of the world. They are well known to produce natural metabolites like steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics which having broad spectrum of biological activities that includes anticancer, antiviral, antibacterial, antidiarrhoeal. So far, more than 200 bioactive metabolites have been isolated from true mangroves
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of tropical and subtropical populations. The mangrove plants are known be to rich in classes of polyphenols which already known for its strong cytotoxic effects, hence mangrove plants could be an excellent resource for drug hunting (Satapathy et al., 2013).

A. marina contains important chemical constituents with potential medicinal properties and it has been used traditionally for treatment of ulcers and skin diseases. Several studies have confirmed that A. marina having anticancer, antiviral, antibacterial and antifungal activities (Itoigawa et al., 2001; Khafagi et al., 2003; Zhu et al., 2009). A. marina is having rich source of polyphenolic compounds and many plant derived flavonoids have phenolic compounds were evaluated to induce apoptosis and tested for sensitization of TRAIL in different cancer cell lines. (Jacquemin et al., 2010). Flavonoid like Luteolin and kaempferol were already reported for sensitization of TRAIL in lung and cervical cancers (Yan et al., 2012; Ham et al., 2014). The same compound was also isolated from leaves of A. marina (Sharaf et al., 2000; Jia et al., 2004; Momtazi-borojeni et al., 2013). Still many such compounds need to be evaluated, therefore in the present investigation A. marina was chosen for isolating the TRAIL deciphering compounds.

1.13. Endophytic fungi

Endophytic fungi reside within the plant tissues for at least a part of their life time without causing any manifestation of disease and having symbiotic relationship with their host plants (Kusari and Spiteller, 2012a). During this association, none of the interacting partners is discernibly harmed, and the individual benefits depend on both the interacting partners. They can synthesis various bioactive chemicals
including alkaloids, terpenoids, steroids, quinones, lignans, phenols and lactones. Those compounds are playing vital role in host plant growth, stress and disease resistances. During the long period of co-evolution, a friendly relationship was gradually set up between endophytic fungus and its host plant. The host plant can supply plenteous nutrient and easeful habitation for the survival of its endophytes. On the other hand, the endophytes would produce a number of bioactive compounds for helping the host plants to resist external biotic and abiotic stresses, and benefiting for the host growth in return (Rodriguez et al., 2009).

Generally, these compounds are structurally novel and potential to use in modern medicine for numerous diseases (Kusari et al., 2012b). Occasionally, endophytes that produce host plant secondary metabolites with therapeutic value or potential have been discovered. Some examples includes paclitaxel, also known as Taxol which is known as an effective anticancer drug (Stierle et al., 1993), podophyllotoxin (Eyberger et al., 2006), deoxypodophyllotoxin (Kusari et al., 2009), camptothecin and structural analogs (Puri et al., 2005).

Since the "gold" bioactive compound paclitaxel (taxol) discovered from the endophytic fungus *Taxomyces andreanae* in 1993, many scientists have been increasing their interests in studying fungal endophytes as potential producers of novel and biologically active compounds. In the past two decades, many valuable bioactive compounds with antimicrobial, insecticidal, cytotoxic and anticancer activities have been successfully discovered from the endophytic fungi (Zhang et al., 2006).

✓ Tumor necrosis factor-α (TNF-α) related apoptosis inducing ligand (TRAIL) is a potent stimulator of apoptosis. It is more specific to cancer cells. Hence, TRAIL was considered as a promising therapeutic candidate for treatment of various cancers.

✓ TRAIL is an important immune effector molecule involving tumor immune surveillance and elimination of developing tumors.

✓ In preclinical trials, recombinant forms of TRAIL showed strong activity against TRAIL-sensitive tumor cells in vitro and in vivo. Early-phase clinical trials using recombinant TRAIL indicate that these agents can be delivered safely.

✓ But clinical observation showed that the vast majority of human cancers are resistant to apoptosis induction by TRAIL. Because of the resistance it has failed to give anti-tumor responses in clinical trial.

✓ Due to down regulation of TRAIL-Rs and up regulation of anti-apoptotic protein developed the TRAIL resistance in cancer cells. Resulting inactivation of the TRAIL pathway and/or escape from TRAIL-mediated and immunosurveillance.

✓ The current challenge is to overcome TRAIL resistance and make the cancer cells sensitive to TRAIL. Recent studies raveled that combinational therapy TRAIL and therapeutic agents, especially naturally occurring polyphenolic can restored tumor cell sensitivity to rhTRAIL.
HPV16 caused nearly 76.7% of cervical cancer and 74,000 women die. Its nearly 1/3rd of the global cervical cancer deaths.

HPV oncogeneic protein E6 directly involved in the development of TRAIL resistant by interacting with FADD and pro-caspase 8, both E6 and E7 involved in deregulations of apoptosis process at various molecular events.

1.15. **Hypothesis tested**

1. Do mangrove and its endophytic fungi have TRAIL deciphering compounds?

2. Can polyphenolic or other compounds from mangrove sensitize the TRAIL in HPV16 infected cervical cancer cell line SiHa?

3. What could be the synergic effect of TRAIL and mangrove derived compound in SiHa cell line?

4. What could be the effect of mangrove derived compounds on HPV 16 oncogenic proteins E6 and E7?

In line with the above hypothesis, the objectives were chosen.