Cancer refers to any one of a large number of diseases characterized by the development of abnormal cells that divide uncontrollably and have the ability to penetrate and destroy normal body tissue. Prostate cancer (PCa) is abnormal, uncontrolled growth of cells that results in the formation of a tumor in the prostate gland. Almost all PCas are adenocarcinomas in nature. Prostate cancer cells may spread (metastasize) from the prostate to other parts of the body, especially the bones and lymph nodes. The three most common prevalent cancers among males in USA are prostate cancer (43%), colorectal cancer (9%) and melanoma (8%) (De Santis et al., 2014). Prostate cancer is the most frequently diagnosed cancer and the sixth leading cause of cancer related death in men, accounting for 14% of the total new cancer cases and 6% of the total cancer deaths in males in 2008 worldwide (Jemal et al., 2011). Relative to PCa rates in India, the rates are more than 20 times higher in US whites, more than 10 times higher in US Asian Indians/Pakistanis, seven times higher among UK South Asians and twice as high among Singapore Indians (Rastogi et al., 2008). The symptoms of PCa include; difficulty in urinating, painful ejaculation, erectile dysfunction, hematuria and other symptoms (Talcott et al., 1998). Advanced therapeutic interventions have resulted in a decline in the mortality rate of PCa in many developed countries, including Australia, Canada, United Kingdom, United States, Italy and Norway (Baade et al., 2009; Bray et al., 2010). In contrast to the trends in developed countries, incidence and mortality rates are rising in several Asian, Central and Eastern European countries (Jemal et al., 2011).

Prostate cancer patients with high risk are recommended to undergo surgery, radiation, and/or hormone therapies. Although these treatments are efficient in general, some patients have recurrence, at the stage recent treatment options are mostly
chemotherapy. Initially PCa is androgen dependent and responds to androgen deprivation therapy (ADT); however, in advanced stages PCa becomes androgen independent. A complex series of molecular events such as oncogene activation, tumor suppressor gene inactivation, apoptosis evasion, intratumoral androgen production, aberrant androgen receptor (AR) activation and increased growth factor production lead to the development of androgen independence (Isaacs, 1994).

Growth factors are naturally occurring substances, important for regulating a variety of cellular processes such as cell growth, proliferation, and differentiation. Among various growth factors such as Insulin like growth factor - I (IGF-I), Transforming growth factor (TGF), Vascular endothelial growth factor (VEGF), the Epidermal growth factor (EGF) plays a critical role in cancer cell proliferation, differentiation, migration, invasion and angiogenesis. Increased levels of growth factors and its receptors especially IGF and EGF have been observed in many types of cancer including PCa (Borugian et al., 2008). EGF mediates its action by binding to its receptor EGFR, which is a tyrosine kinase receptor. The epidermal growth factor receptor (EGFR; HER1) is a proto-oncogene that encodes a 170 kDa transmembrane protein (Gullick, 2001). EGFR belongs to HER family of tyrosine kinase receptors. EGFR plays a critical role in tumor growth and the prostate tissue becomes more susceptible to the growth-promoting actions of EGF family growth factors during androgen withdrawal. Progression from androgen-responsive tumor to hormone-refractory carcinoma is a multistep process, usually accompanied by the upregulation of growth factor receptors, their ligands and downregulation of tumor suppressor genes (Ware, 1999; Djakiew, 2000). EGFR ligands, such as EGF, HB-EGF (human binding-EGF) and TGF, are expressed in the prostate and prostatic carcinomas (Elson et al.,
1984; Freeman et al., 1998). The major EGF/EGFR signaling pathways include PI3K/Akt and MAPK kinase (MEK)/extracellular-related kinase (ERK) (Singh and Harris, 2005). EGF mediated Akt activation triggers a cascade of responses, from cell growth and proliferation to survival and motility that drive tumor progression (Vivanco et al., 2007). Upon activation of growth factor receptors, Akt family members become phosphorylated on two residues (Thr308 and Ser473) by the phosphoinositide-dependent kinase 1 (PDK1) and the mammalian target of rapamycin rictor (mTOR) complex respectively (Engelman, 2009). Akt regulates at least four different but interacting pathways: cell survival, growth, metabolism and progression through the cell cycle (Engelman, 2009). Akt phosphorylates and inactivates glycogen synthase kinase-3β (GSK-3β). GSK-3β phosphorylates and inactivates crucial cell cycle regulators and transcription factors, including β-catenin, cyclin D1 and c-Myc (Liu et al., 2009; 2014). Nuclear Factor κ B (NFκB) is a key regulator of genes involved in cell activation and proliferation (Grilli et al., 1993). NF-κB activity is normally regulated through its cytoplasmic sequestration by specific inhibitors including inhibitory κB (IκB) and related proteins.

Mitogen-activated protein kinases also known as MAP kinases are serine/threonine/tyrosine-specific protein kinases belonging to the CMGC (CDK/MAPK/GSK3/CLK) kinase group. There is substantial evidence validating the importance of Raf and ERK in cancer progression (Shields et al., 2000). The importance of this pathway in oncogenesis was first suggested by the initial identification of Raf as potent retrovirus oncogenes (Schreck and Rapp, 2006). Raf kinases phosphorylate and activate the MEK1 and MEK2 dual-specificity protein kinases. MEK1/2 (MAPKK) then phosphorylates and activates the ERK1/2, MAPKs.
Activated ERKs can translocate to the nucleus, where they phosphorylate and regulate various transcription factors, such as E26 transformation-specific (ETS) transcription factors family (e.g., Elk-1), ultimately leading to changes in gene expression (Zuber et al., 2000; Schulze et al., 2001). Inhibition of tyrosine kinase signaling pathways provides therapeutic advantage against PCa metastasis (Festuccia et al., 2005). Therefore, inhibiting the activation of growth factor receptors, especially EGFR, may be a promising strategy for the treatment of PCa. A novel therapeutic approach should be to target the epidermal growth factor receptor (EGFR), which is often overexpressed in many tumors and regulates proliferation, apoptosis, angiogenesis, tumor invasiveness, and metastasis (Baselga, 2002; Grandis and Sok, 2004).

During the course of tumor progression, cancer cells undergo dynamic changes that result in detachment of cells from their original tissue with acquisition of highly motile and invasive phenotype. Epithelial–mesenchymal transition (EMT) is the hallmark of this change, during which the expression of adhesion molecules that regulate the interaction of cancer cells with the extracellular matrix (ECM) and their neighboring cells changes (Huber et al., 2005; Thiery and Sleeman, 2006). A common feature of cancers of epithelial origin is increased expression of N-cadherin and decreased expression of E-cadherin; a major component of adheren junctions that brings about hemophilic contact between neighboring cells. This molecular switch with the loss of E-cadherin and the \textit{de novo} expression of N-cadherin is termed cadherin switching (Hazan et al., 2004). This phenomenon is correlated with the acquisition of invasiveness and metastatic potential of the cancer cells. Studies have shown a high correlation between loss of E-cadherin, the gain of vimentin and tumor invasiveness in cancer cells and patient tumors (Qiao et al., 2008). A down regulation of E-cadherin
most frequently results from transcriptional repression, mediated by zinc finger, forkhead domain and basic helix-loop-helix (bHLH) transcription factors like Snail, Slug and Twist. The expression of Snail, Slug and specific bHLH transcription factors have been implicated in cell survival and acquired resistance to chemotherapy (Cho et al., 2007; Yin et al., 2007). N-cadherin promotes tumor cell survival, migration and invasion. The high-level expression of N-cadherin is often associated with a poor prognosis. In addition, various experimental evidences suggest that N-cadherin is a potential therapeutic target in cancer treatment (Mariotti et al., 2007).

Intercellular adhesion molecule-1 (ICAM-1, also called CD54) is an inducible surface glycoprotein that mediates cell–cell adhesion (Zimmerman and Blanco, 2008). ICAM-1 is crucial for trans-endothelial migration of leukocytes from the capillary bed into the surrounding tissue (Duperray et al., 1997), but it may also facilitate migration of other cell types (Yang et al., 2010). ICAM-1 plays a key role in breast and lung cancer cell invasion (Grothey et al., 1998). The knockdown of ICAM-1 expression reduces the invasiveness of breast cancer cells (Chen et al., 2011). Since, ICAM-1 plays a critical role in tumorigenesis; disruption of ICAM-1 may prove useful in preventing cancer cell metastasis. Vascular cell adhesion molecule-1 (VCAM-1) is a cell surface molecule that mediates cell adhesion. When aberrantly expressed in breast cancer cells, VCAM-1 mediates distinct tumor-stromal interactions that are unique to lung and bone microenvironments and facilitate metastasis to these sites (Ruco et al., 1996; Lu et al., 2011). Little is known about the function of VCAM-1 in other cancers. However, VCAM-1 expression has been reported in gastric, renal carcinomas and in PCa (Lu et al., 2011), where it might play roles similar to those reported in breast cancer (Shin et al., 2006).
Selectins (P-selectin & E-selectin) mediate cell adhesion and are involved in cancer cell metastasis. Selectins facilitate tumor cell adhesion during cancer cell migration (Franziska et al., 2014). Franziska et al. (2014) reported that selectins mediate lung cancer metastasis. The inhibition of selectin expression correlates with inhibition of metastasis. Similarly, it has been reported that anti-E-selectin-agents, impairs lung metastasis. The up-regulation of selectins in PCa, together with its limited expression in normal body tissues, makes it a potential therapeutic target. Borsig et al. (2002) reported that cancer metastasis is impaired in P-selectin deficient mice.

Metastasis is the process by which a tumor cell leaves the primary tumor, travels to a distant site via the circulatory system, and establishes a secondary tumor. In order to metastasize, cancer cells must invade through the basement membrane and the extracellular matrix (ECM). Proteolysis of the ECM is an important step in metastasis and the process is associated with the upregulated production and activity of several ECM degrading proteases. The matrix metalloproteinases (MMPs) comprise a family of structurally related, zinc dependent endopeptidases that are capable of degrading the protein components of the ECM, basement membrane and release the growth factors from ECM stores (Catania et al., 2007). MMP activity is regulated by specific inhibitors, the tissue inhibitors of MMP (TIMPs).

Apoptosis is a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. The ability to modulate the life or death of a cell is recognized for its immense therapeutic potential (Norbury and Hickson, 2001). Inefficient apoptosis is considered as one of the hallmarks of tumorigenicity (Dowsett et al., 2006). Moreover, induction of apoptosis is
an important target for cancer therapy (Fesik, 2005). There are two major apoptosis signaling pathways. The death receptor (extrinsic) pathway and the mitochondria (intrinsic) mediated pathway. The extrinsic pathway is initiated by cell surface expressed death receptors of the tumor necrosis factor superfamily. One of the central pathways of apoptosis is initiated by cytokines, such as tumor necrosis factor-α (TNF-α), Fas ligand (FasL) and tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL) (Ashkenazi and Herbst, 2008). Once the receptor is activated by Fas ligand, it recruits intracellular adaptor proteins and forms scaffolding complexes (Youle and Strasser, 2008). The complexes recruit one or more members of the caspase family of cell death protease, classically caspase-8. This activated initiator caspase cleaves downstream effector caspases, in particular caspase-3.

The intrinsic pathway is initiated by anticancer drugs, growth factor withdrawal, hypoxia, or via induction of oncogenes. These stimuli induces permeabilization of the outer mitochondrial membrane and releases apoptogenic factors like cytochrome c from the mitochondrial intermembrane space into the cytosol. This release of cytochrome c into the cytosol triggers caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9 - containing apoptosome complex (Riedl and Shi, 2004). Caspase-3 then cleaves a large number of intracellular substrates, now numbering ~400, which culminate in the morphological changes of apoptosis (Taylor and Nicot, 2008).

A voluminous literature suggests that an increase in consumption of fruits and vegetables are a relatively easy and practical strategy to reduce significantly the incidence of cancer. The beneficial effect is mostly associated with the presence of phytochemicals (Flavonoids) in the diet. Various epidemiological studies have
demonstrated that increased consumption of fruits and vegetables have decreased the incidence of certain forms of cancer (Kandaswami et al., 1992; Gullett et al., 2010). Flavonoids possess a wide range of biochemical and pharmacological effects. Many flavonoids have been shown to possess anticancer properties. The predominant mechanism of their anticarcinogenic action is by their capacity to scavenge free radicals (antioxidant activity), enzyme inhibition and/or antiproliferative activity (Nirmala et al., 2011).

Quercetin (3,3’,4’,5,7-pentahydroxyflavone) is a member of the flavone family and is found in many foods, including vegetables, tea, fruit, and wine (Hertog et al., 1996). It is marketed as a diet supplement with anti-histamine, anti-inflammatory, antiviral, immunomodulatory and anti-oxidant properties (Ross and Kasum, 2002). It also possesses anti-tumor, anti-fungal, vasorelaxation activity on hippocampal neurons (Pu et al., 2007). It scavenges superoxide in ischemia-reperfusion injury, protects against oxidative stress induced by UV light, spontaneous hypertension secondary biliary cirrhosis, bacterial lipopolysaccharide and carcinogenesis (Huk et al., 1998; Erden Inal and Kahraman, 2000; Yang et al., 2004).

Quercetin inhibits the proliferation of various cancer cells (Choi et al., 2001, 2009; Kuo et al., 2002; Ong et al., 2004; Choi et al., 2009; Kim et al., 2010). Quercetin also enhances TRAIL-induced apoptosis in PCa cells via increased protein stability of death receptor 5 (Jung et al., 2010b). Quercetin has been demonstrated to inhibit tumor growth and angiogenesis by targeting VEGF-R2 regulated AKT/mTOR signaling pathway in PCa cells (Pratheeshkumar et al., 2012). Quercetin combined with EGCG in vitro demonstrated enhanced inhibition of cell proliferation in PCa cells (Wang et al., 2014). Our previous studies also showed that quercetin induces apoptosis and arrest
cell cycle in PCa cells (Vijayababu et al., 2005; 2006; Senthilkumar et al., 2010, 2011). Quercetin down regulates the cell survival, proliferative and an anti-apoptotic protein thereby prevents PCa, by acting as a chemopreventive agent in Sprague Dawley rats (Sharmila et al., 2014 a, b).

However, the role of quercetin on EMT signaling molecules in PCa remains unclear. So the present study is aimed to investigate the effect of quercetin on EGF-mediated cell survival, proliferation, migration and invasion of PCa cell line (PC-3).