INTRODUCTION

The plants have gained considerable importance due to their potential health benefits that are attributed to the presence of myriads of antioxidants. Several studies have shown that the intake of plant food rich in antioxidants improve the quality of health as it controls many degenerative processes and can effectively reduce the incidence of cancer and heart diseases (Arabshahi and Urooj, 2007). The recovery of antioxidants from plant sources is typically adapted via different extraction techniques taking into consideration their chemical nature and uneven distribution in the plant matrix. The aromatic and medicinal plants are the sources of diverse nutrients and non-nutrient molecules. A wide number of medicinal plants display antioxidant, antitumor, anticancer, antiproliferative and antimicrobial properties that can defend the human body against the cellular oxidation reactions as well as pathogens. Therefore, it is important to categorize different types of medicinal plants for their potential to combat against diseases (Mothana and Lindequist, 2005; Bajpai et al., 2005; Wojdylo et al., 2007).

Plants derived drugs are used since prehistoric times in the treatment of cancer. Cancer has been regarded as a group of diseases and its incidence is now rapidly rising worldwide. The main reason for the development of different diseases is oxidative stress. The free radicals generated from molecular oxygen are involved in the prognosis of various ailments. Excessive generation of free radicals also cause the cellular damage causing cytotoxicity (Sun and Peng, 2008). Most of the anticancer drugs presently used in chemotherapy are known to be cytotoxic to normal cells as well as cause undesirable side effects. Therapeutically, the effective doses of many anticancer drugs generate the oxidative stress in normal tissues of heart and brain (Chen et al., 2007). A number of promising natural constituents, polyphenolic compounds derived from plants such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or pre-clinical development. However, ethnopharmacological observations in the past have resulted in the discovery of several important drugs for example, taxol derived from yew. Likewise, combretastatin was isolated from the bark of the South African...
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tree *Combretum caffrum* (Combretaceae). It is effective against the treatment of colon, lung and blood cancers. It is also estimated that this molecule is the most cytotoxic phyto molecule isolated till date (Shoeb, 2006).

The medicinal plants are getting more attention than ever throughout the world because they have countless benefits to society or indeed to all mankind, especially in the line of medication and pharmacological studies. The beneficial properties of plants lie in the presence of bioactive phytochemicals that produce specific physiological action on the human body (Akinmoladun *et al.*, 2007). A variety of plants contain ordinary antioxidants as bioactive constituents such as alkaloids, essential oils, saponins, flavonoids, phenolics, tannins, curcumin and terpenoids (Prakash *et al.*, 2007 and Edeoga *et al.*, 2005). In recent times, the use of natural antioxidants such as phenolic substances (flavonoids, phenolic acids and tocopherols) in food along with preventive and therapeutic medicine is gaining much appreciation. Such natural substances are supposed to show evidence of anticarcinogenic activity and offer diverse health promoting effects because of their antioxidant attributes (Eloff, 1999; Sultana and Anwar, 2008; Sultana *et al.*, 2007). The increased intake of plant foods correlates with decreased rate of death from dreaded diseases (Johnson, 2001). Approximately, 60% of the commercially available antitumor agents are of natural origin (Cragg *et al.*, 1997). The free radical scavenging potential of polyphenols is primarily due to their redox properties that make them to play the role as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin (Rice Evans *et al.*, 1995; Prior *et al.*, 2005; Lopez *et al.*, 2007; Ciz *et al.*, 2008; Gebicka and Banasiak, 2009).

The antioxidant competence among foodstuffs and natural products is tested by the use of variety of tests. Presently, worldwide there is not a perfect method that can compute the antioxidant capability of all samples precisely and quantitatively (Frankel and Finley, 2008; Prior *et al.*, 2005). Herbs have been explored for their use in many areas along with nutrition, medicine, flavoring, beverages, cosmetics etc. The intake of fresh fruits, vegetables and tea rich in natural free radical scavengers has been linked with prevention of cancer and heart diseases (Willcox *et al.*, 2004). The polyphenolic compounds having flavonoid nucleus have been isolated from plants and shown to have
free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and antiinflammatory action (Frankel, 1995; Pourmorad et al., 2006; Omale and Okafor, 2008). Extensive epidemiological studies have shown that dietary intake of flavonoids reduce the risk of coronary heart diseases and certain cancers (Hung et al., 2004; Puupponen et al., 2001; Tripoli et al., 2007). Many plants are considered to be rich sources of flavonoids which are used, not only to preserve food but also makes part of a healthy diet (Justesen and Knethsen, 2001). Dietary flavonoids are considered to be powerful antioxidants as compared to vitamins C and E (Sokol et al., 2006).

Antioxidants may play vital role in the metabolic disorders. In plants, polyphenol production and accumulation is generally moved in response to biotic and abiotic stresses such as salinity (Naczk and Shahidi, 2004; Navarro et al., 2006). Most of the studies have shown that the amount of polyphenols and antioxidant activities depend on biological factors as well as edaphic and environmental (temperature, salinity, water stress and light intensity) conditions (Lisiewska et al., 2006). The amount of UV-B radiations falling on the earth surface depend on many factors viz. ozone, clouds, aerosols and albedo (Bais et al., 2007). The level of UV-B radiations also change with season and altitude (Mckenzie et al., 2001; Pfeifer et al., 2006). Indeed, the highest levels of UV-B radiations in European ecosystems are recorded in summer season, especially in June and the lowest in the winter season (Seckmeyer et al., 2008). In addition, total irradiance to UV-A radiations increase on an average 9% and 11% per 1000 m of elevation and UV-B radiations increase by 19% (Blumthaler et al., 1992). As a result, the altitudinal migration of plant species in response to global warming will expose them to higher doses of UV radiations, especially UV-B (Penuelas et al., 2007; Parolo et al., 2007).

Ultraviolet-B radiations are the most energetic component of sunlight reaching the earth surface and is one of the photo oxidative stress factor for plants that affects their physiology and morphology (Stratmann, 2003). Among plant responses to the increase in UV-B radiations, there are changes at the morphological (increase in leaf thickness and specific leaf weight or decrease in plant height) and biochemical levels (synthesis of phenolic compounds) (Caldwell et al., 2007; Paoletti, 2005). It is well known that a general response to enhanced levels of UV-B radiations is the biosynthesis
of phenolic compounds (Tiitto et al., 2005), since they can help to filter the UV radiations avoiding or minimizing the penetration of UV-B into internal tissues (Krauss et al., 1997).

Natural therapies like the use of plant derived products and Traditional Chinese Medicine (TCM) in the cancer treatment may reduce adverse side effects (Dai et al., 2011; Shynu et al., 2011; Wang et al., 2012). The natural products of plant origin exhibited low or almost no toxicity to normal tissues and are effectively used as cancer chemotherapeutic drugs. Therefore, more attention is being paid to search for new antitumor agents from plant sources (Sigstedt et al., 2008; George et al., 2010). The selection of new phytochemical against cancer is required for many reasons as firstly, with time, some plant species with antiproliferative activity may undergo extinction before they are ever studied. Secondly, new products are required to reduce many problems linked with cancer treatment as the most prevalent ones include drug resistance, toxicity, hair loss, nausea, loss of appetite and the low specificity of currently available cytotoxic drugs (De et al., 2009). Thirdly, with the development of new technologies, high throughput screening of plants for their anticancer potential is urgent where molecules isolated from them are proving to be an important inhibitors of key proteins that have regulatory effects on tumor cell cycle progression (Amin et al., 2009). The majority of plant species with bioactive phytochemicals have not yet undergone chemical, pharmacological and toxicological studies.

There has been a great curiosity to discover the natural products from medicinal plants for their use in cancer therapeutics. A countless number of medicinal plants are known to have biochemical constituents with anticancer properties. The chemical metabolites of natural origin that exhibit anticancer properties can serve as potential lead compounds in drug designing (Raina et al., 2014). According to Cragg and Newman (2000), more than 50% of the drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them. Chemoprevention involves the use of pharmacological and whole plant extracts to arrest or reverse the cellular and molecular processes of carcinogenesis (Mehta et al., 2010). Moreover, the synergistic effects of plant metabolites offer higher efficacy during chemoprevention regimens (Guilford and Pezzuto, 2008).
Botanicals are thought of offering strong therapeutic efficacy with minimal side effects, since most of their effects are from a mixture of active molecules acting at the same time. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Among the 2,50,000 higher plant species on earth, more than 80,000 plants have medicinal value. As, India is one of the biodiversity centers with the presence of over 45,000 different plant species. Nearly, 20,000 plants have good medicinal value and only 7500 species are used for their medicinal activities by traditional communities. It is well established that plants have always been useful source for the occurrence of anticancer compounds (Stankovic et al., 2011; Reddy et al., 2003; Guo et al., 2010). About 60% of presently used anticancer chemotherapeutic drugs (vinblastine, vincristine) are derived from plant resources (Cragg and Newman, 2005; Tan et al., 2006).

The tumor promotion has been reported to be inhibited by natural phytochemicals via retarding the genotoxic effects, increased free radical scavenging and anti-inflammatory activity, inhibition of cell division and regulation of apoptosis and signal transduction pathways (Soobrattee, 2006). It has been found that many plant derived chemotherapeutic drugs kill cancer cells by enhancing apoptosis (Bhalla et al., 1993; Takano et al., 1993; Hertzberg et al., 1989; Fearnhead et al., 1994). Anticancer drugs kill cancer cells by inhibiting growth or division at some point in their life cycles. The plants exert their cytotoxic effect by down-regulating the antiapoptotic genes such as COX-2, iNOS, TNF-α, Bcl-2 and up-regulation of proapoptotic genes such as Bax, p21, p53, cytochrome C and caspase. Interference of NF-kB activity downstream by cryptolepis/cryptolepine may be oppressed in cancer treatment or improve sensitivity of cancer cells to chemotherapy and radiotherapy (Nataru et al., 2014).

Apoptosis initiation is regarded as preferred cancer treatment approach where apoptotic mechanism is ideally targeted in pharmaceutical development of anticancer drugs. It can be induced via two different ways, one is intrinsic pathway which is usually initiated by chemical agents or irradiation. The other is extrinsic pathway initiated via the binding of death ligands to the death receptors. These two pathways can converge at mitochondria to promote mitochondrial leakage and the release of pro-apoptotic factors to trigger caspase dependent cell death or directly cause cell death.
(Desagher and Martinou, 2000; Du et al., 2000; Wang and Ki, 2001). Caspases are very important for the initiation and execution of programmed cell death (Cohen, 1997). Some of them such as caspase-8 and 9 are “initiators” of this process while others like caspase-3, 6 and 7 are “executioners”. These execution caspases such as caspase-3 can break many important proteins and cause the disassembly of cell structure and DNA fragmentation which results into cell death (Cryns and Yuan, 1998; Thornberry and Lazebnik, 1998).

Several studies in literature have led to the discovery and development of new active ingredients from natural molecules or derivatives and several of these compounds are used today in clinical practice. The use of naturally occurring molecules in the treatment of cancer has greatly contributed to the improvement of the therapeutic efficacy of drugs used today in cancer chemotherapy. A large number of anticancer agents are being certified on the basis of their potential to change one or more molecular events. The identification of herbs effective in unfolding the underlying molecular mechanisms could lead to the discovery of potential drugs for prevention and treatment of cancer. Keeping this in view, the present study was planned to assess the antioxidative and anticancer potential of *Acacia nilotica* by using leaves extract/fractions of summer and winter seasons.

*Acacia nilotica* (Prickly acacia or Vachellia nilotica, Sant tree, Gum Arabic tree) is 5 to 20 meter in height having dense spheric crown and stems along with branches which are dark to black colored. The bark is fissured having gray-pinkish slash and have a reddish low quality gum. The origin of name *Acacia* means “spiny” which is typical feature of the species. *A. nilotica* is resident to Africa and Indian subcontinent and grows in abundance and develops into small trees. *A. nilotica* has a wealth of medicinal uses, mainly for treating cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma (Ambasta, 1994). The wood was used by ancient Egyptians to make sculptures and furnishings. In West Africa, the bark or gum is used for the treatment of cancers (ear, eye and testicles). It has also been traditionally used in the treatment of various infectious ailments such as pneumonia, cold and urinary tract infections (Bargali and Bargali, 2009). The plant is rich in phenolics which mainly consist of condensed tannin and phlobatannin, gallic acid, protocatechuic acid,
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pyrocatechol, (+) - catechin, (-) epigallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate (Kirtikar and Basu, 1975; Raghavendra et al., 2006). The scientific classification of A. nilotica is as:

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<tr>
<th>Binomial name</th>
<th>Vachellia nilotica</th>
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<tr>
<td>Kingdom</td>
<td>Plantae</td>
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<tr>
<td>Order</td>
<td>Fabales</td>
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<tr>
<td>Family</td>
<td>Fabaceae</td>
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<tr>
<td>Subfamily</td>
<td>Mimosoideae</td>
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<tr>
<td>Genus</td>
<td>Acacia</td>
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<td>Species</td>
<td>A. nilotica</td>
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Although, some studies on the bioactivities of bark and pods of A. nilotica are there in literature but the antioxidant and antiproliferative activities of leaves collected in different seasons viz. summer and winter are not available. With this in view, the following objectives were framed:

1. Preparation of extract/fractions:
   I. Extraction of plant material collected in two seasons by employing maceration method
   II. Qualitative estimation of extract/fractions for phytochemicals
   III. Quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) by spectrophotometer
   IV. Quantification of different polyphenols by using ultra high pressure liquid chromatography (UHPLC)

2. In vitro antioxidant studies
   I. Hydrogen and electron donating assays (Molybdate ion reduction assay, Cupric ion reducing assay, ABTS radical cation decolorization assay and β-carotene linoleic acid assay)
II. Radical scavenging assays (Superoxide anion scavenging assay, Lipid peroxidation assay and DNA nicking assay)

3. In vitro antiproliferative studies

I. In vitro antiproliferative studies against a number of human cancer cell lines by using 3-(4,5-Dimethylthiasol-2-yl)-2,5,-diphenyltetrazolium Bromide (MTT) assay

4. Mechanistic studies

I. Morphological examination of apoptotic cells
   ❖ Confocal microscopy by using DAPI stain
   ❖ Scanning electron microscopy (SEM)

II. Cell cycle analysis by flow cytometer

III. Measurement of mitochondrial membrane potential (MMP) by spectrofluorometer

IV. Measurement of reactive oxygen species (ROS) by spectrofluorometer

V. Spectrophotometric analysis for the assessment of Caspase-3 activity