Health benefits and effects of plant derived antioxidants

INTRODUCTION

Plant derived antioxidants especially, the phenolics have gained considerable importance due to their potential health benefits. Several epidemiological studies have shown that the intake of plant food containing antioxidants improve the quality of health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardiovascular diseases (Arabshahi and Urooj, 2007). The revitalization of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry 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 which can protect the human body against both cellular oxidation reactions and pathogens. Therefore, it is important to characterize different types of medicinal plants for their potential to fight against diseases (Mothana and Lindequist, 2005; Bajpai *et al* ., 2005; Wojdylo *et al* ., 2007).

Plants have a long history of use in the treatment of cancer. In many instances, “ cancer” is undefined or reference is made to conditions such as hard swellings, abscesses, calluses, corns, warts, polyps or tumors. Cancer has been regarded mainly as a group of diseases. The incidence of various forms of cancer is now rapidly rising worldwide. The main reason for the development of different diseases is oxidative stress. The free radicals formed from molecular oxygen have been implicated in the pathogenesis of various diseases. Excessive production of free radicals also results in cellular

damage causing cytotoxicity (Sun and Peng, 2008). Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells that leads to unwanted side effects. Therapeutically, the effective doses of many anticancer drugs turn out the oxidative stress in normal tissues such as heart and brain (Chen *et al* ., 2007). A number of promising agents such as flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol are in clinical or pre-clinical development. An abundant types of bioactive compounds have been isolated from plant sources. Combretastatin was isolated from the bark of the South African tree *Combretum caffrum* (Combretaceae). It is effective against the cancer of colon, lung and blood. It is also estimated that this molecule is the most cytotoxic phyto molecule isolated so far (Shoeb, 2006).

The world is loaded with natural and limited medicinal plants and getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medication and pharmacological studies. The medicinal properties of plants lie in bioactive phytochemical constituents that produce definite 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 foods 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 privileged ingestion of plant foods correlates with minor jeopardy of death from these diseases (Johnson, 2001). Roughly, 60% of the commercially accessible antitumor and antiinfective agents are of natural origin (Cragg *et al.* , 1997). The antioxidant activity of polyphenols is mainly due to their redox properties which allow them to act 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 samples 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 studied for their use in many areas together with nutrition, medicine, flavoring, beverages, cosmetics etc. The intake of fresh fruits, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases (Willcox *et al.* , 2004). Flavonoids are group of polyphenolic compounds with known properties such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and antiinflammatory action have been isolated from plants (Frankel, 1995; Pourmorad *et al* ., 2006; Omale and Okafor, 2008). Extensive epidemiological studies have indicated an inverse relationship between dietary flavonoids intake and 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 excellent sources of flavonoids that can be used, not only to preserve food but also contribute to a healthy diet (Justesen and Knethsen, 2001). Dietary flavonoids are considered to be even more powerful antioxidants than 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 polyphenolics 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 reaching the earth surface depend on many factors viz. ozone, clouds, aerosols and albedo (Bais *et al* ., 2007). The level of UV-B radiations also changes seasonally and altitudinally (Mckenzie *et al* ., 2001; Pfeifer *et al* ., 2006). Indeed, the highest levels of UV-B in European ecosystems are recorded in summer, especially in June and the lowest in the winter season (Seckmeyer *et al* ., 2008). In addition, total irradiance and UV-A radiations increase on average 9% and 11% per 1000 m of elevation and UV-B radiations increase 19% (Blumthaler *et al* ., 1992). Consequently, 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 filter UV radiations avoiding or minimizing 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). Medicinal plants and natural products of plant origin are rich source of cancer chemotherapy drugs and exhibited low or almost no toxicity to normal tissues. Therefore, more attention is being paid to search for new antitumor agents from natural products (Sigstedt et al., 2008; George et al., 2010). The selection of new natural compounds against cancer is necessary for many reasons as firstly, with time, some plant species with anticancer activity may suffer extinction before they are ever studied. Secondly, new products are required to counteract many difficulties associated with cancer treatment as the most common of which include drug resistance, toxicity, excessive 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, the revival of collection of plants and high throughput screening for their anticancer potential is urgent where molecules isolated from plants are proving to be an important source of novel inhibitors of the

action of key proteins that have regulatory effects on tumor cell cycle progression (Amin *et al* ., 2009). The majority of plant species have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds.

There has been an distinction curiosity for the discovery of medicinal plants and derived natural products for increasing cancer therapeutics. An infinite number of medicinal plants are known to have biochemical constituents with anticancer properties. The chemical metabolites of natural origin that acquire anticancer properties can serve as potential lead compounds in drug designing (Raina *et al* ., 2014). According to Cragg and Newman (2000), over 50% of the drugs 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 prevent 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).

The plant derived natural products are gaining importance to cure various disease status. The various side effects of allopathic drugs and development of resistance to presently used drugs have lead to increased emphasis on the use of plant materials as a source of medicines for a large variety of human ailments. Incidentally, plants and herbs are persistently being studied for the identification of novel therapeutic agents. 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 values 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 derivative from plant resource (Cragg and Newman, 2005; Tan *et al* ., 2006). Moreover, traditionally, plants passed empirical testing against specific diseases and demonstrated that they are well tolerated in humans.

The preventive mechanisms of tumor promotion by natural phytochemicals range from the inhibition of genotoxic effects, increased antioxidant and antiinflammatory activity, inhibition of cell proliferation and modulation of apoptosis and signal transduction pathways (Soobrattee, 2006). The mechanism of possible plants induced anticancer effect includes cell cycle distribution and apoptosis. It has been found that many plant derived chemotherapy drugs kill cancer cells by promoting apoptotic cell death (Bhalla *et al* ., 1993; Takano *et al* ., 1993; Hertzberg *et al* ., 1989; Fearnhead *et al* ., 1994). Anticancer drugs destroy cancer cells by stopping growth or multiplication at some point in their life cycles. The plants exert their cytotoxic effect by down-regulating the antiapoptotic genes such COX-2, iNOS, TNF-α, Bcl-2 and up-regulation of proapoptotic genes such 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 activated through two different pathways, one is intrinsic pathway which is usually initiated by chemical compounds or irradiation. The other is extrinsic pathway activated by 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 activation and execution of apoptosis (Cohen, 1997). Some of them such as caspase-8 and 9 are ‘‘ initiators’’ of the apoptotic process while others like caspase-3, 6 and 7 are ‘‘ executioners’’. These execution caspases such as caspase-3 can cleave many important proteins and cause the disassembly of cell structure and DNA fragmentation which ultimately leads to 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 wide number of new chemopreventive agents are being accredited based on their capability to modify one or more molecular events. The invention of effective herbs and elucidation of their underlying molecular mechanisms could lead to the development of prospective 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 and tumors (ear, eye and testicles). It has also been traditionally used in the treatment of various infectious diseases 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, 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:

Binomial name	*Vachellia nilotica*
Kingdom	Plantae

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Order	Fabales
Family	Fabaceae
Subfamily	Mimosoideae
Genus	*Acacia*
Species	*A. nilotica*

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:

    1. Extraction of plant material collected in two seasons by employing maceration method
    2. Qualitative estimation of extract/fractions for phytochemicals
    3. Quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) by spectrophotometer
    4. Quantification of different polyphenols by using ultra high pressure liquid chromatography (U-HPLC)

2. *In vitro* Antioxidant studies

1. Hydrogen and electron donating assays (Molybdate ion reduction assay, Cupric ion reducing assay, ABTS radical cation decolorization assay and ß-carotene linoleic acid assay)
2. Radical scavenging assays (Superoxide anion scavenging assay, Lipid peroxidation assay and DNA nicking assay)

3. *In vitro* antiproliferative studies

1. *In vitro* antiproliferative study 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

1. Morphological examination of apoptotic cells

- Confocal microscopy by using DAPI stain
- Scanning electron microscopy (SEM)

2. Cell cycle analysis by flow cytometer
3. Measurement of mitochondrial membrane potential (MMP) by spectrofluorometer
4. Measurement of reactive oxygen species (ROS) by spectrofluorometer
5. Spectrophotometric analysis for the assessment of Caspase-3 activity