Chapter 2

Literature Review
Chapter 2

PART A

Molecular approaches towards development of purified natural products and their structurally known derivatives as efficient anti-cancer drugs: Current trends

Background

Several natural products and their derivatives either in purified or structurally identified form, exhibit immense pharmacological and biological properties, some of them showing considerable anticancer potential. Although the molecular mechanisms of action of some of these products are yet to be elucidated, extensive research in this area continues to generate new data that are clinically exploitable. Recent advancement in molecular biology, high throughput screening, biomarker identifications, target selection and genomic approaches have enabled us to understand salient interactions of natural products and their derivatives with cancer cells vis-à-vis normal cells. In this review I highlight the recent approaches and application of innovative technologies made to improve quality as well as efficiency of structurally identified natural products and their derivatives, particularly in small molecular forms capable of being used in “targeted therapies” in oncology. These products preferentially involve multiple mechanistic pathways and overcome chemo-resistance in tumor types with cumulative action. I also mention briefly a few physico-chemical features that compare natural products...
with drugs in recent natural product discovery approaches. I further report here a few purified natural products as examples that provide molecular interventions in cancer therapeutics to give the reader a glimpse of the current trends of approach for discovering useful anticancer drugs.

**Introduction**

Compounds with biological activities, and products derived from natural sources, e.g., plants, animals and microorganisms are defined broadly as “natural products” that constitute the major sources of chemical diversity, in purified or structurally identified form. These compounds can also be classified as crude therapeutic formulations, semi-synthetic natural products, natural product derived compounds, herbal medicines and complementary and alternative medicines (CAM). Many drugs used recently for therapeutic applications are complex natural products or natural product derivatives (Salvador et al., 2012; Clardy and Walsh, 2004). Cancer remains a major public health issue as more than one million people are diagnosed with cancer each year (Howlader et al., 2012). Development of resistance against chemotherapy, toxicity and side-effects necessitates the search for relatively non-toxic drugs or natural products, to wage a more humane war against cancer (Pan and Ho, 2008). However, orthodox approaches for cancer therapy including surgery, radiotherapy and adjuvant therapies are still necessary.

In this review, I will focus more on the following aspects: (a) evaluation of purified natural products and their further strategic up-gradation for more specific use in future anticancer drugs; (b) developmental application of innovative technologies useful in tissue-specific targeting, chemo-resistant cancer types and (c) recent trends of molecular approaches towards targeted cancer therapeutics.

But at the onset, I will briefly dwell upon the features that compare natural products with drugs. The architectural determinants that highlight comparison between natural products and synthetic drug molecules in pharmacological applications have been outlined below.
Both the natural products and synthetic molecules share high chemical diversity, biochemical specificity, molecular mass, number of chiral centers, molecular flexibility and distribution of heavy metals suitable for therapeutic applications (Henkel et al., 1999). Compared to synthetic medicinal agents, natural products contain more number of carbon, hydrogen, oxygen and less nitrogen and other elements. Further, the molecular rigidity is greater in natural products as compared to synthetic drugs and other combinatorial drugs (Feher and Schmidt, 2003). Many useful natural products have high polarity rendering them greater water solubility, an important feature in maintaining pharmacokinetics guidelines of oral administration in terms of better adsorption, distribution, metabolism and elimination from body (Butler, 2008). Natural product structures are evolutionarily selected to interact with a wide variety of proteins and other biological targets for specific purposes. The ability of natural products to bind a variety of protein domains and folding motifs leads to modulate or inhibit protein-protein interaction, making these molecules behave as effective modulators of cellular processes such as immune responses, signal transduction, mitosis and apoptosis. By virtue of increased size and binding affinity to gene products natural products provide effective scaffolds for the biological function (Peczuh and Hamilton, 2000). Most of the natural products show several key properties as specified in the “Lipinski’s rule of five” (Lipinski et al., 1997) appropriate for oral administration. Thus, these criteria clearly point out that natural products can play a significant role in the selection and design of the most effective drug in respect of its specific application.

Recent approaches towards molecular cancer therapies with purified natural products

Cancer is a disease of uncontrolled proliferation and growth of cells at inappropriate times and locations in the body. When cells acquire mutations that affect the regulation of
cell division, they undergo unlimited multiplications to form tumors (Hanahan and Weinberg, 2011); these proliferating cells consequently transform into malignant ones and invade other tissues as a result of metastasis.

Recent drug discovery programme seeks for rapid screening, hit identification and hit-to-lead generation. In these circumstances, traditional natural product development programs which are based on extract-library screening, bioassay-guided isolation, structure elucidation and subsequent product scale up processes, are lagging behind as compared to the approaches that have largely been utilized in the synthetic chemical process. However, the high throughput screening methods in combination with approaches of human genetics and genomics towards functional validation of target regions, especially by over expression or knockdown by RNA-interference in transgenic animals and model organisms (Benson et al., 2006) are yielding interesting results. This outcome is also creating interest in pursuing studies on natural products as a source of chemical diversity and lead generation. New approaches with natural products for drug designing and their further strategic up-gradation for specific use in molecular cancer therapeutics are outlined below.

**Structurally identified and purified natural product libraries**

Natural product samples are stored as “crude extract library” for further screening purposes. The crude extract is further concentrated, fractionated and purified to yield essential biologically active component(s) and stored as “pure compound library” (Koehn and Carter, 2005). With the advancement of technology such as HPLC-mass spectrometer, and natural product database resources [e.g., Dictionary of Natural Products, SciFinder and Antibase] identification of known compounds, if any, after purification process became simplified (Strege, 1999). In those cases where the criteria of biological activity and selectivity persist, preliminary structure activity relationship helps in de novo complex structure determination and additional
optimization procedure proceeds onward following medicinal chemistry guidelines of drug development. Due to time limiting process of separation, isolation and purity check of crude mixture collection; the present focus is more on keeping “semi purified chemical compounds” as the base stock; but the interest is still persisting towards making the “fully purified compounds” readily available for use when needed (Baker et al., 2007).

**High throughput screening–identification of genomic markers of natural product sensitivity in cancer cells**

The exercise of automated testing of huge collections of compounds (natural products as well as synthetic molecule libraries) for any specific biological targets (inhibitors, activators, cell surface receptors etc.) to identify any potential drug is commonly known as high throughput screening. Human tumor derived cell line models maintained and propagated either in culture or in mice xenografts and more recently, genetically engineered mouse models of human tumorigenic applications are very effective to the discovery and evaluation of potential new anticancer agents (Sharma et al., 2010). National Cancer Institute 60 platform established in 1986, introduced the concept of high throughput cell based profiling. NCI 60 panel comprises approximately 6-9 human tumor derived cell lines, representative of each cancer type used to capture frequencies of drug response (Adams, 2001). Researchers at the Cancer Chemotherapy Center of the Japanese Foundation for Cancer Research established a panel of 39 human tumor derived cell lines in 1999, that included a subset of the National Cancer Institute 60 cell lines and additional gastric cancer cell lines (based on prevalence in Japanese population). This platform is also successful in identifying anticancer agents as well as cancer biomarker identification (Shiwa et al., 2003). Considering the conserved nature of 20 different tissue origins with equal representation, an ideal human tumor cell line profiling panel should consist of 2000-6000 cell lines. With this goal in mind, a human cell line platform, with as broad representation as possible, has recently been established (2006), which currently includes 1200 cell lines
panel. The Center for Molecular Therapeutics 1000 platform profiled 127 candidates of anticancer agents and interrogated for cytostatic and cytotoxic activity against approximately 700 human tumor derived adherent cell lines (corresponding to around 70,000 drug-cell lines pairing as of May 2009 report) (Sharma et al., 2010). Genetically defined cancer subsets, irrespective of the tissue of origin is also associated with response to drug sensitivity. Thus current standard practice in medical oncology follows patient’s genotypic information for therapy (McDermott et al., 2007, http://www.ox.ac.uk/media/news_stories/2013/130325.html). Due to genomic heterogeneity present in tumors in different human populations, heterogeneity of cell types in tumor cell populations is also likely to have an important role in drug sensitivity (Rosen and Jordan, 2009). Therefore mutation analysis data for these cell lines with drug sensitivity are now being integrated in an effort to better understand the genomic determinants of drug response to various cancer therapeutic approaches. Validation and prioritization of the best targets for the targeted cancer therapy is also very critical. There is certainly no shortage of potential drug targets. More than 350 cancer genes have been catalogued (Futereal et al., 2004) for drug sensitivity. With these cell-line based approaches of drug sensitivity, a very recent work dealing with screening of 130 drugs (under clinical and preclinical investigation) has identified 64 cancer biomarkers successfully (Garnett et al., 2012).

**Lead generation- a critical step of optimization for drug design**

The next major step after target selection is lead generation. Lead generation is an optimization procedure. This is accomplished through chemical modification by employing structure activity relationship and structure-based designing (if structural information of biological proteins as well as chemical structure of the compound are known) to improve potency, reduce off target activities and develop pharmacokinetics of the compound to be tested as therapeutic drug molecule. Chemical biology and structural biology data are combined together to reach the goal. The chemical biology process involves,
phenotypic screening for lead generation and for lead optimization the process incorporates biomarker selection and probing selectivity. On the other hand, the structural biology includes library designing and virtual screening for lead generation, followed by the process of designing potency and understanding selectivity for lead optimization (Collins and Workman, 2006). Phenotypic screening method in intact cells or in organisms such as *Caenorhabditis elegans* or zebrafish embryos is applicable to target identification and lead generation (Clemons, 2004) for some small molecule’s target screening that are incompatible with *in vitro* biochemical assays and many molecular targets to be probed simultaneously in the cellular environment. Some imaging based high content screens interrogate the phenotype directly at the molecular level (Lundholt et al., 2005). Sensitive biophysical techniques such as nuclear magnetic resonance and X-ray diffraction can detect the weak bindings of much smaller compounds. The finding of p38 mitogen activated protein kinase inhibitor is one such example of lead generation (Gill et al., 2005).

Combining biochemical high throughput screening and biophysical methods, protein–ligand co-crystallography is a powerful and efficient lead generation strategy (Card et al., 2005). Computational chemistry provides empirical parameters that describe appropriate physiochemical properties of drugs that are routinely incorporated into new compound library design (Vieth et al., 2004). Examples of some natural products that enter into clinical trial after these high throughput screening and lead generations include camptothecins, vinca alkaloids, and epothilones (Mann, 2002).

**Structure based approaches for drug discovery**

Computational chemistry is an important ally that is contributing to a great extent towards screening strategies. Some structural motifs that confer promiscuous nonspecific activity are associated with toxicity can be eliminated in this process. The various approaches require generation of huge compound libraries. Identification of several protein classes such as kinases and Heat Shock Protein 90 are examples of structure based designing approaches.
The structure based approach with natural products, such as geldanamycin and radicicol, which are bound to N-terminal ATPase domains of Heat Shock Protein 90 revealed a unique folding pattern in the ATP binding site (Roe et al., 1999). The drug discovery programme was conducted by Developmental Therapeutics Programme of the National Cancer Institute, USA, which maintains some important databases and tools for cancer therapeutics. Links to these and additional databases can be found at http://dtp.nci.nih.gov/. Molecular pharmacology and drug discovery strategies make use of three important databases: (A) activity pattern database; (B) molecular structural features of tested compound database; (C) possible targets or modulators of activity in the cell database (Weinstein et al., 1997). “Patterns of activity” of any compound often indicate similarity in mechanism of action, mode of resistance and molecular structure and this can be analyzed using “COMPARE” tools. Patterns of activity could predict a compound mechanism of action (Weinstein et al., 1997). The information intensive approach to molecular pharmacology shakes hands with recent approaches in cancer therapeutics.

**Emerging strategies of using interferons in anti-cancer drug development programme**

Type 1 interferons, which comprise a group of cytokines, are currently used as a novel therapeutic strategy in the treatment of cancer. Interferon-α demonstrates response through Epidermal growth factor dependent ras/MEK1/erk and adenylate cyclase/cAMP signaling events in cancer cells. The AKT/NFκβ pathway mediated survival response has also been shown when cells were exposed to Interferon-α. Combinatorial effect of Interferon-α either with single or multiple pathway inhibitors might be useful in chemotherapeutic approach with its low concentration. As for example, when epidermal growth factor receptor inhibitor gefitinib was used along with IFN-α, effective co-operative antitumor activity (Caraglia et al., 2013) was noted. Interferon-α induces both apoptosis and a counteracting epidermal growth factor
dependent survival response in cancer cells (Lamberti et al., 2007).

**Aptamer as cancer therapeutic agent**

Aptamers are non immunogenic, short oligonucleotides or peptides representing a valid alternative to antibodies that target specific cancer cell surface proteins in clinical diagnosis and therapy. Cell “Systematic Evolution of Ligands by Exponential enrichment” approach is the method of choice to develop nuclease or proteinase resistance aptamers for target specific delivery in physiological conditions. Wu et al (2010) reported self-assembled aptamer-micelle nano structure that achieved selective and strong bindings to targets; low in off rate once bound with target, rapid recognition ability, dual drug delivery ability and with *in vivo* drug delivery applications as its property. Li et al (2011) and Esposito et al (2011) devised independently anti-epidermal growth factor receptor aptamer for cancer cell death. Cancer cells that are resistant to epidermal growth factor receptor inhibitors such as gefitinib and cetuximab showed significant response when combined treatment with cetuximab and RNA-aptamer were used as therapy (Esposito et al., 2011). Application of DNA aptamer as molecular probe for human papilloma virus associated cervical cancer cells is also reported (Graham and Zarbl, 2012).

**Genomic construction vs. natural products in cancer therapy**

Dietary habitats, food and lifestyle factors can influence development of cancer risk to an individual by targeting the genetic susceptibility. Several of the most common susceptible genetic variations are single nucleotide polymorphisms like CYP1A1, CPY1A2, CYP2D6, GSTM1 and NAT2) which cause differences in metabolic or detoxification activities (Perera, 1996). Incidence of increased lung cancer risk has been correlated with variant forms of CYP1A1 that catalyse the oxygenation of polyaromatic hydrocarbons; polyaromatic hydrocarbons are present in cigarette smoke and therefore smoking increases the cancer risk. Other genes such as CYP2D6 (acts on an unknown substrate,
possibly tobacco-specific nitrosamines), and GSTM1 (detoxifies reactive, electrophilic compounds such as polyaromatic hydrocarbons) (Perera, 1996) also have similar functional role in increasing risk of cancer. The relative prevalence and distribution of such polymorphisms in populations that may affect individual responses to both risk and protective factors can be targeted by antioxidants and other naturally occurring dietary constituents for cancer therapeutic strategy.

**Targeted cancer therapy and signalling events**

The term “targeted cancer therapeutics” describe small molecular agents that are not only rationally designed but also acts on disease causing oncogenic targets. Various well defined phenotypic hallmark traits of cancer (Hanahan and Weinberg, 2011) can be good targets for cancer therapy; these include (a) self sufficiency in growth signals, (b) evasion of apoptosis, (c) limitless replicative potentials, (d) angiogenesis, (e) tissue invasion and (f) metastasis. Oncogene products themselves and their downstream signalling proteins are also suitable targets (e.g. mTOR, PI3 kinase, RAS-RAF-MEK-ERK pathway). In addition, protein chaperone (e.g. heat shock protein 90), chromatin regulation (e.g. histone deacetylase) and autophagy can also provide valuable drug targets (McDonald et al., 2006; Minucci and Pelicci, 2006; Lamy et al., 2013).

Therapeutic approaches that can target more than one of these mechanisms gain preference and attention for drug formulation in cancer therapy. Treatment of cancer by targeting any one of the above mentioned acquired capabilities by administration of one or more chemical entities attracts interest of the recent workers on cancer therapy.

**Driving cells into apoptosis- the process of “programmed cell death” as drug target**

The ability of tumor cell population to expand in number is determined not only by the rate of cell proliferation but also by the rate of cell death. Programmed cell death—“apoptosis” represents a major source of
this attrition. Members of the Bcl-2 family of proteins, whose members have either pro-apoptotic (Bax, Bak, Bid, and Bim) or anti-apoptotic (Bcl-2, Bcl-XL, Bcl-W) functions, act in part by governing mitochondrial death signalling through cytochrome c release. The p53 tumor repressor protein can elicit apoptosis by up-regulating expression of pro-apoptotic Bax in response to sensing DNA damage; Bax in turn stimulates mitochondria to release cytochrome c. The ultimate effectors of apoptosis include an array of intracellular proteases termed as caspases (Thornberry and Lazebnik., 1998).

**Innsensitivity to growth signals- “cell-cycle” as molecular targets**

Cancer cells must evade anti-proliferative signals if they are to prosper. Much of the circuitry that enables cells to respond to anti-growth signals is associated with cell cycle clock, specially the component governing the transition of the cell through the G1 phase of its growth cycle. Cyclin dependent kinases with their different cyclin subunits are catalytic components that regulate key switches throughout cell cycle. Regulation of transcription, differentiation, nutrient uptake and other functions of cells are tightly controlled by these protein kinases (Massague, 2004).

**Limitless replicative potential- “telomeric-DNA” as drug target**

Telomerase maintenance is evident in virtually all types of malignant cells (Shay and Bacchetti., 1997). 85%-90% of them succeed in doing so by up-regulating expression of the telomerase enzyme gene, which adds hexa-nucleotide repeats onto the ends of telomeric DNA (Bryan and Cech, 1999), while the remainder have invented a way of activating mechanism termed alternative lengthening of telomerase which appears to maintain telomerase through recombination based in terchromosomal exchange of sequence information (Bryan et al., 1995). By one or the other mechanism, telomerase is maintained at a length above a critical threshold, and this in turn permits unlimited multiplication of descendant
cells. Both mechanisms seem to be strongly suppressed in most normal human cells in order to deny them unlimited replicative potential.

**“Angiogenesis” signals as therapeutic target**

The oxygen and nutrients supplied by the vasculature are crucial for cell function and survival, obligating virtually all cells in a tissue to reside within a capillary blood vessel. Once a tissue is formed, the growth of new blood vessels the process of angiogenesis is transitory and carefully regulated. The angiogenesis initiating signals are exemplified by vascular endothelial growth factor and acidic and basic fibroblast growth factors. Each binds to transmembrane tyrosine kinase receptors displayed by endothelial cells (Veikkola and Alitalo, 1999). Tumors appear to activate the angiogenic switch by changing the balance of angiogenesis inducers and countervailing inhibitors. Triphala and Its active constituent chebulinic acid are found to be natural inhibitors of vascular endothelial growth factor (Lu et al., 2012).

**Targeting tissue invasion and “metastasis”**

Sooner or later during the development of most types of human cancer, primary tumor masses spawn pioneer cells that move out, invade adjacent tissues, and hence travel to distant sites where they may succeed in founding new colonies. The capability for invasion and metastasis enables cancer cells to escape the primary tumor mass and colonize new terrain in the body where, at least initially, nutrients arise as amalgams of cancer cells and normally supporting cells conscripted from the host tissues. Matrix metalloproteinase 15 is identified as an anti-apoptotic factor in cancer cells (Abraham et al., 2005).

**Targeting self sufficiency in growth signals**

Normal cells require mitogenic growth signals before they can shift from a quiescent state into an active proliferative state. No type of normal cell can proliferate in the absence of such stimulatory signals. On the other hand cancer cells act by mimicking
normal growth signalling events in one way or another to achieve the characteristics of self regulation to promote the growth without depending on others. Cancer cells acquire the ability to synthesize growth factors to which they are responsive, creating a positive feedback signalling loop often termed autocrine stimulation (Hanahan and Weinberg, 2011). The production of platelet derived growth factor and tumor growth factor-α by glioblastomas and sarcomas, respectively, are two illustrative examples (Hanahan and Weinberg, 2011). Cancer cells can also switch on both ligand activated growth factor receptors and pro-growth integrins engaged to extracellular matrix component to activate SOS-Ras-Raf-MAP kinase pathway (Aplin et al., 1998). Ras proteins are present in structurally altered forms that enable them to release a flux of mitogenic signals into cells, without ongoing stimulation by their normal upstream regulators (Medema and Bos., 1993).

**Targeting chaperone**

The proteasome is a catalytic complex that is found in both nucleus and cytoplasm of eukaryotic cells. The function of the proteasome is to degrade or process intracellular proteins- e.g. mediators of cell cycle progression (cyclins), apoptosis (caspases, Bcl2, and NFκβ). Proteasome inhibitors are the choice of recent cancer therapeutic targets (Adams, 2004). Members of heat shock protein family exhibit a good target for molecular cancer therapy. Heat shock protein 90 is over expressed in many human cancers. X-ray crystallography data of heat shock protein 90 revealed conserved water molecules in the active sites of hydrogen bonding network that are the potent target for natural product as well as synthetic compounds. Natural products such as geldanamycin, radicicol, and novobiocin are good examples of heat shock protein 90 target. Among these, geldanamycin analogues entered into the stage of clinical trials (McDonald et al., 2006).
Targeting histone deacetylase

Re-programming of cellular metabolism is a key event during tumorigenesis. The histone deacetylase SIRT6 acts as a tumor suppressor that regulates aerobic glycolysis in cancer cells (Sebastian et al., 2012). Histone deacetylases are currently being tested in phase I/II clinical trials (Minucci and Pelicci, 2006) for cancer drug targets.

Autophagy as cancer drug targets

Another very important targeted cancer therapy in resistant chemotherapy is “autophagy”. Autophagy is a multistep lysosomal degradation process in which a cell destroys long lived proteins and damaged organelles. Drugs targeting autophagy induced cell death also sensitize apoptosis. The cross talk between apoptosis and autophagy is mediated via PI3K/Akt/mTOR Pathway (Sun et al., 2012). Some natural products such as curcumin, resveratrol, paclitaxel, oridonin, quercetin and plant lectin may lead to cancer cell death by regulating some core autophagic pathways involved in Ras-Raf signalling, Beclin-1 interactome, BCR-ABL, PI3KCI/Akt/mTOR, FOXO1 and p53 signalling (Zhang et al., 2012). A recent report of Lamy et al (2013) discusses autophagic control in multiple myeloma by caspase 10 and provides an in-depth idea about the important molecular target in cancer therapeutics.

Role of some purified and structurally defined natural products in targeted cancer therapy

Utilization of natural products as effective drugs for cancer therapy is still an elusive goal due to the lack of proper bio-target identification, specificity aspect, lack of understanding of the molecular mechanism of action, and the signalling pathways involved. I will mention hereunder the functional role and the suggested molecular mechanisms of action and different signalling pathways of a few natural products in their purified form (Figure 2.1) in cancer therapy as examples of how this kind of approach is possible (Table 2.1).
Figure 2.1 Chemical structures of some important natural products used in cancer therapy include- (1) berberine, (2) palmatine, (3) chelidonine, (4) chelerythrine, (5) sanguinarine, (6) coptisine, (7) lycopodine, (8) lycorine, (9) curcumin, (10) scopoletin, (11) camptothecins, (12) epothilones and (13) geldanamycin to use in future anticancer agent.
Table 2.1 Selected structurally identified and purified natural products causing cancer cell death following specific signaling

<table>
<thead>
<tr>
<th>Natural products</th>
<th>Molecular targets</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>Modifies cysteine 179 of IKBα kinase with suppression of NFκβ and induce apoptosis. G2/M cell cycle arrest associated with increased expression of P53, Wee 1 and CDk 1 and decreased expression of Cyclin B. Androgen-insensitive (DU145 and PC-3) and androgen-sensitive (LNCaP) prostate cancer cells also show cell cycle arrest by targeting cdki-cdk-cyclin cascade. Metastasis inhibition on nasopharyngeal carcinoma 5-8F cells by targeting Rho-Kinase mediated Ezrin phosphorylation at threonine 567. Antitumor effect on malignancies such as esophageal cancer, hepatoma, lung carcinoma, leukaemia, uterine cancer, prostate carcinoma and gastric carcinoma. Significant DNA binding and anti-tumor activity against HL-60 leukaemia cells.</td>
<td>Pandey et al., 2008; Lin et al., 2006; Mantena et al., 2006; Tang et al., 2009; Izuka et al., 2000; Mitani et al., 2001; Letasiova et al., 2006; Kuo et al., 1995</td>
</tr>
<tr>
<td>Sanguinarine</td>
<td>ROS mediated induction of apoptosis. NFκβ mediated differential apoptotic response. Induction of cyclin kinase inhibition p21/WAF1 and p27/ KIP1, down-regulation of cyclin E, D1, D2 and cyclin-dependent kinase 2, 4, 6. Effective against multidrug resistance human cervical cells.</td>
<td>Malikova et al., 2006; Ahmad et al., 2000; Adhami et al., 2003; Ding et al., 2002</td>
</tr>
<tr>
<td>Chelerythrine</td>
<td>Induction of cell cycle and apoptosis in cancer cells.</td>
<td>Malikova et al., 2006; Chan et al., 2003</td>
</tr>
<tr>
<td>Lycopodine</td>
<td>ROS mediated MMP depolarization, release of Cytochrome-c and activation of caspase-3 which are events closely involved in apoptosis and G1 cell cycle arrest</td>
<td>Mandal et al., 2010</td>
</tr>
<tr>
<td>Compound</td>
<td>Activity</td>
<td>Reference</td>
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<tr>
<td>------------</td>
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<tr>
<td>Palmatine</td>
<td>Inhibition of reverse transcriptase, topoisomerase I and II and structure activity relationship are suggested to be probable reason for antitumor activity.</td>
<td>Sethi, 1983</td>
</tr>
<tr>
<td>Chelidonine</td>
<td>Transcriptional down regulation of catalytic subunit of telomerase (hTERT) and senescence induction was reported in HepG2 cells.</td>
<td>Noureini et al., 2009</td>
</tr>
<tr>
<td>Ukrain</td>
<td>Bcl-2 controlled mitochondrial signalling events leading to apoptosis</td>
<td>Habermehl et al., 2006.</td>
</tr>
<tr>
<td>Coptisine</td>
<td>Cytotoxic on human tumor colon cell line (LoVo) and colon carcinoma cell line (HT 29) and less potent on murine leukaemia (L-1210). It was effective on the LoVo cell resistant to doxorubicin, and murine leukaemia cell line (L-1210) resistant to cisplatin.</td>
<td>Colombo et al., 2001</td>
</tr>
<tr>
<td>Lycorine</td>
<td>Potential use of lycorine showed to treat leukaemia cells resistant to apoptotic stimuli. Potential use as therapeutic agent has recently led to studies into the production of the synthetic compound highlighting it as a potential lead for drug development.</td>
<td>Lamoral-Theys et al., 2010; Jones et al., 2009; El Tahchy et al., 2010; McNulty et al., 2009</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Many health benefits such as antioxidants, antibacterial, anti-inflammatory, neuroprotective and anti carcinogenic effects have been investigated.</td>
<td>Esatbeyoglu, 2012</td>
</tr>
</tbody>
</table>
Outlook and future challenges- application of innovative technologies in cancer therapy

A better understanding of the key patho-physiological differences between differential interaction of natural compounds and their derivatives in cancer cells and their counterparts in non-malignant cells will undoubtedly be instrumental for increasing the level of selectivity of targeted anticancer agents. The discovery and development of potent anticancer agents has been greatly hampered due to lack of suitable preclinical model to check the efficacy of candidate agents. To bridge the gap, innovation in xenografts models and genetically engineered models of human tumorigenesis would play an important role in developing cancer therapeutic strategy. Applications of a few incoming innovative technologies are mentioned below (Figure 2.2).

The importance of tumor microenvironment, three dimensional aspects of solid tumors and the response to therapy at these conditions have pushed forward to build such models more precisely in vitro. In fact 3D cell cultures are believed to be an alternative model of in vivo counterparts. Examples of 3D culture systems include, multilayer cell systems, matrix embedded 3D cultures, hollow fibre-based approaches, ex vivo tumor cultures and multi-cellular tumor spheroids (Yamada and Cukierman, 2007). Similarly, one of the potential issues associated with xenografts as models of drug efficacy is inappropriate dosing. With the improvement of animal models for human tumors such as orthotopic models, metastatic models, autochthonous models and genetically engineered cancer models, identification of clinically useful agents is likely to improve in future (Gopinathan and Tuveson, 2008). Some tumor derived cell lines are remarkably similar to that of the primary tumors from which they originated, implying a valid genetic surrogate of tumors in vivo. Examples include: 51 breast cancer cell lines (Neve et al., 2006); 101 melanoma derived cell lines (Lin et al., 2008); and 84 non small cell lung carcinoma cell lines (Sos et al., 2009). Another important aspect is the
Figure 2.2 Some of the emerging strategies that can employ in cancer drug development

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selection and identification of biomarkers in cancer therapeutics. A huge amount of information on genomic, transcriptomic, proteomic and epigenomic data obtained from different ‘systems’ platform make the job as difficult as “finding a needle in bush”. System biology approaches help to find out a meaningful correlation of drug sensitivity data to establish molecular signatures or cancer biomarkers; e.g. yielding an expression signature of 13 genes that are associated with the sensitivity of 48 breast cancer cell lines to the polyamine analogue PG-11047 (an investigational clinical compound).

A major limitation of drug formulation in cancer lies in its drug resistance property. Use of drug-sensitive cancer derived cell lines as models provides an alternative to solve this issue. Drug resistant clones are selected after a continuous exposure over a period of time in vitro. Genetic information which include mutation data from re-sequencing studies, gene expression information from microarrays, identifying gene copy number variations etc. facilitate the identification of drug sensitivity (Drexler et al., 2008). Multi drug resistance is one of the main reasons of treatment failure in cancer. Multi drug resistance protein such as ATP binding cassette transporter family mainly controls the multi drug resistance mechanism by cellular efflux and decreases the effectiveness of chemotherapeutic agents. Inhibitors of multi drug resistance family proteins are developed but are toxic in nature. Chen (2010) evaluated the importance of nuclear receptor, named as pregene X receptor, in controlling drug metabolism and drug clearance. On the other hand interferon family of peptides are playing emerging role in cancer therapeutics but the anti tumor activity of Interferon- α is limited due to activation of tumor resistance mechanism. Interferon-β is emerging as considerably more potent therapeutic agents in cancer through induction of apoptosis and cell cycle arrest to overcome tumor resistance machinery (Caraglia et al., 2009). Recently, Troiani et al(2013) described a number of antibody based therapeutic strategies including development of nanotech devices, combinatorial agents blocking alternative escape pathways, targeting epidermal growth factor
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receptor and immune system to overcome drug resistant cancer cell populations. A recent research approach worth mentioning in this context is on the development of nanoparticles loaded drug delivery system. Kaur and Tikoo (2013) reported that poly lactic-co-glycolic acid encapsulated gefitinib exhibit enhanced anti-cancer effects. They also observed that the nano-particle covered drug acted as a ‘histone acetyltransferase’ rather than ‘tyrosine kinase inhibitor’ (gefitinib in its free form acts more as tyrosine kinase inhibitor). Patra et al. (2012) described important aspects of background theory of design parameters for optimizing gold nanoparticles for cancer therapy. Bhattacharyya et al. (2011) reported poly lactic-co-glycolic acid encapsulated synthetic coumarin to have enhanced anti-cancer effect as revealed from their studies on cellular toxicity and biodistribution in cancer cells, both in vitro and in vivo. Bisphosphonates, a synthetic analogue of naturally occurring pyrophosphate compounds, reported to have ability to induce apoptosis in cancer cells, are candidates which need to be tested in nano-form for possible ability to deliver the drugs in bone tumors (De Rosa et al., 2013) more successfully. Another novel cell delivery system which is emerging for target specific gene therapy is by using peptide nucleic acid. The antisense technology combined with reporter tag in duplex form or reporter tag- peptide nucleic acid in triplex form facilitates rapid, sensitive, selective detection technique as a biosensing platform for detecting target molecule (Yildiz et al., 2012).

Methodological drawbacks

Establishment of a causal relationship between a particular therapeutic application and cancer is difficult. The occurrence of this chronic disease might have been the outcome of any point mutation (due to environmental/behavioural/food/lifestyle stress) in chromosomal DNA that successfully replicate generation after generation in cell. Physiological diets of laboratory animals are too different from humans that it needs to justify the translation of results obtained in these studies (Wicki and Hagmann, 2011). Controlled experiments conducted to
find out the effects of any therapeutic strategies are of two types: (a) observational studies and (b) cohort studies. Both types of studies are flawed due to hidden biasness introduced in terms of self chosen groups (Ioannidis, 2005). Randomised controlled trials are more reliable approach in this aspect of conducting hypothesis generating experiments because in this case, individuals are randomly assigned to treatment groups (Martinez et al., 2008).

Although much of the phenomena and causes of cancer have now been elucidated, and a lot of strategies have been made to find out the foolproof remedy, unfortunately none has succeeded so far to prevent cancer from occurring or curing it when it has advanced to a certain limit (Figure 2.3). Some of the major reasons for failure in cancer drugs development have been suggested as due to poor pharmacokinetics, insufficient therapeutic activity, toxicity, and poor bioavailability (Kola and Landis, 2004). With all these difficulties and obstacles in way natural products as a source of new drugs serving mankind for decades (Newman and Cragg, 2007).

Figure 2.3 methodological drawbacks that pull down developmental approaches against cancer are
PART B

Strategies of molecular interventions with some medicinal plant products: oxidative stress and cancer prevention

Background

Cells in humans are constantly exposed to a variety of oxidizing agents from naturally occurring processes, environmental stimuli or pollutants and of lifestyle stress or activities. The key factor in a biological system is to maintain a balance between oxidants and antioxidants to sustain optimal physiological conditions. Imbalance of the process leads to oxidative stress which causes oxidative damage to the biomolecules such as lipids, proteins, and most importantly DNA, resulting in an increased risk of diseases like cancer. Epidemiological studies have shown that effects of various medicinal plants and their bioactive components can perform a great role in cancer chemoprevention. This part of the literature review summarizes the current knowledge on some medicinal plants and their phytochemicals in targeted cancer therapy and discusses the molecular approaches that can possibly be made with them in order to achieve some effective anti-cancer drug formulation.

Introduction

Cancer remains a major public health issue that profoundly affects human health as more than one million people are diagnosed each year with this disease (Howlader et al., 2012). In this report, Howlader (2012) also estimated that approx 1,638,910 people (about 848,170 men and 790,740 women) are at the risk of being diagnosed with cancer and about 577,190 of them are likely to die, that too only in the USA. This surely is a situation of horror and needs careful
attention as to how best we can manage this problem.

Because of modernization and explosive growth of various sectors of industry, including the automobile industry, pollutants are constantly being added to the environment with an increasing load of carcinogens. These environmental carcinogens from various sources contribute to an individual’s total burden of oxidative stress and invade the antioxidant defense mechanisms of human body and other biological systems. The resulting damage leads to genetic mutations with the outcome of carcinogenesis/malignancy (Greenwald and McDonald, 1999). The accumulation of oxidative damage has been implicated in both acute and chronic cell injuries including possible participation in the formation of cancer. Cellular oxidative stress can modify intercellular communication, protein kinase activity, membrane structure and function, and gene expression, and result in modulation of cell growth.

Many medicinal plants are reported to contain antioxidants in adequate amount that give them the ability to protect them from oxidative assaults or physiological stress generated by ROS, and therefore, have made them subject of further investigation if they could also be utilized for reducing cancer risk (Diplock, 1996; Cozzi et al., 1997). Many epidemiological studies have consistently shown that adequate consumption of foods of plant origin, such as fruits, vegetables, whole grains, legumes, nuts, seeds, tea along with a change in related lifestyle can decrease the risk of developing various cancers (Krebs-Smith and Kantor, 2001). In fact, a recent epigenetic research (Colacino et al., 2012) has shown that DNA methylation occurs with the pre-intake of certain medicinal plant phytochemicals, and tumor suppressor effect becomes discernible in head and neck squamous cell carcinomas.

Chemopreventive potentials of naturally occurring medicinal plant as antioxidants and their bioactive phytochemicals are suggested to have effects on cellular signalling molecules as targets. These molecules trigger induction of cellular defense systems to detoxify the agents responsible for
generating the oxidative stress by synthesizing the requisite amount of anti-oxidative enzymes. Further, as an additional measure of protection, inhibition of inflammatory and cell growth signalling pathways is initiated that culminates in progression of the cells into the apoptotic pathway and bringing the cell cycle to a halt (Pan and Ho, 2008).

The major aim of this review is to focus on the anti-oxidative properties of some medicinal plants and their phytochemicals, and to elucidate the molecular approaches (targeting mechanism of oxidative stress and ROS signalling events, mainly) with some of these medicinal plants as antioxidants that can be made for targeted cancer therapy.

**Oxidative Stress and Reactive Oxygen Species (ROS) – the “rusting disorder”**

At an international conference of the World Health Organization (WHO) in the mid-1960s Professor Sanojki, a Russian toxicologist, described certain diseases, the origin of which could be attributed to oxygen toxicity; for an example, rheumatoid arthritis. He termed the phrase- “the rusting diseases”- for all such diseases. The diseases that have been directly implicated to oxidative stress for their origin include cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, Schizophrenia, Bipolar disorder, fragile X syndrome disorder, Sickle Cell anemia, lichen planus, vitiligo, autism, chronic fatigue syndrome and many others (Basu and Temple, 1999).

“Oxidative stress” – definition, sources and chemistry in general

“Oxidative stress” is literally defined as “an imbalance between the ROS generation and its detoxification by biological system leading to impairment of damage repair by cell/tissue (Beliveau and Gingras, 2007). ROS, which include superoxide anion radical (O$_2^-$), singlet oxygen (1O$_2$), hydrogen peroxide (H$_2$O$_2$), and the highly reactive hydroxyl radical (.OH), can be generated due to
naturally occurring processes such as mitochondrial electron transport, exercise, environmental stimuli and pollutants (e.g., ionizing radiation), change in atmospheric conditions (e.g., hypoxia), and lastly because of lifestyle stress (e.g., cigarette smoking, alcohol consumption etc) (Pan and Ho, 2008).

Cellular system has evolved a range of metallo-enzymes and regulatory systems that facilitate interaction between redox metals with O$_2$ using various catalytic pathways, the end products resulting in the generation of free radicals and ROS (Bush, 2000), as shown below:

\[
\text{Fe}^{3+} + \cdot\text{O}_2^- \rightarrow \text{Fe}^{2+} + \text{O}_2 (\text{step 1})
\]

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH} (\text{step 2})
\]

Step 1 + step 2

\[
\cdot\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \text{HO}^- + \text{O}_2
\]

[Fenton reaction]

Apart from the direct effect, ROS generation can also be achieved substantially by disruption of calcium channels with the help of metallo-enzymes (Demuro et al., 2005).

To counter the oxidant effects and to restore redox balance, cells must reset important homeostatic parameters.

**Cancer: definition and the role of oxidative stress**

Cancer is a disease of uncontrolled proliferation and growth of cells at inappropriate times and locations in the body. When cells acquire mutations that abolish regulation of cell division, the cells multiply to form masses that we call tumors (Hanahan and Weinberg 2000) and these cells consequently transform to malignant ones and invade other tissue as a result of metastasis.

There is a growing awareness that oxidative stress plays a role in various clinical conditions including malignant diseases (Apel and Hirt, 2004; Bergamini et al., 2004). The initial process of tumor promotion involves a nonlethal and inheritable mutation in cells by interaction of a chemical with DNA. This mutation confers a growth advantage to that cell. For the mutation to be set around, DNA synthesis must
occur to lock in the mutation. The activation of the carcinogen to an electrophilic DNA-damaging moiety is a necessary step for this stage. ROS are believed to mediate the activation of such carcinogens through hydroperoxide-dependent oxidation that can be mediated by peroxyl radicals (Trush and Kensler, 1991). This occurs with aflatoxin B, aromatic amines, and polycyclic aromatic hydrocarbon dihydrodiols (Trush and Kensler, 1991). ROS or their by-product of lipid peroxidation, MDA, can also directly react with DNA to form oxidative DNA adducts (Chaudhary et al., 1994). The presence of carcinogen-DNA adducts and oxidative DNA adducts generated by chemical carcinogens suggest an important interactive role of ROS in cancer initiation. ROS, therefore, can have multiple effects in the initiation stage of carcinogenesis by mediating carcinogen activation, causing DNA damage, and interfering with the repair of the DNA damage.

Cancer therapeutics by targeting antioxidative mechanisms

Epidemiological studies have consistently shown that intake of medicinal plant phytochemicals are strongly associated with reduced risk of developing chronic diseases such as cancer (Liu, 2004). Chemo-preventive effects elicited by these natural medicinal plants and their photochemical at least in part are due to their antioxidant property, which is the main focus of this review.

Research in cancer therapeutic agents in past few decades has demonstrated that the mechanisms by which antioxidants exhibit their chemo-preventive effects are multiple and complex. The therapeutic strategy employed by any antioxidant against the generation of free radicals causing oxidative stress is either to directly combat and completely eliminate or to convert the ROS to a condition that is less harmful and toxic. In general, the upstream mechanisms include biochemical steps preventing free radial generation, modulating metal-protein
interaction, and promoting normal metal homeostasis while the downstream mechanism involves processes like inflammation and free radical scavenging. To achieve therapeutic success, antioxidants may target different mechanisms and pathways. Among many, some of the probable mechanisms targeting antioxidants are mentioned hereunder.

**ROS scavenging through phase 2 enzymes/ antioxidant activity**

Oxygen radical generation systems e.g., superoxide anion generation by xanthine oxidase, inflammatory cells, tumor promoters, free radical generating systems e.g., benzoyl peroxide produce a range of ROS. Consequently, scavenging ROS is a chemopreventive mechanism; as for example, (i) vitamin C, vitamin E and retinoids (derived from citrus fruits, carrots, green vegetables etc.) provide an integrated antioxidant system, with tissue GSH scavenging ROS and protecting tissues from ROS induced oxidative damage (Parke et al., 1996). (ii) Genistein the active compound derived from soybeans and Curcumin, derived from turmeric belongs to thiol groups such as N-acetylcyesteine are known to react with hydroxyl radicals and phenolic antioxidants (Kensler et al., 1992).

**Reduction of peroxides and repair of peroxidised biological membrane**

The free radicals, peroxides, super oxides generated through oxidative stress can be reduced to their non toxic form to prevent the damage of biological membranes, e.g., the mineral element selenium is essential for this aspect of antioxidant protection and disease prevention, as it is a vital component of the two peroxidase enzymes, GPX, which reduces soluble peroxides and PHGPX, which removes lipid peroxides from biological membranes (Parke et al., 1996).

**Sequestration of iron to decrease ROS formation**

Regulation of metal (iron) formation and their management inside the cell is important in maintaining the balance of oxidative stress, e.g., silicic acid, a ubiquitously distributed
component of cereals and forms complexes with inorganic iron, which enables the safe sequestration of iron in tissues, decreasing its ability to generate ROS, initiates membrane lipid peroxidation, and mobilizes leukocytes (Birchall, 1993).

**Utilization of dietary lipids**

Dietary lipids can produce rapid energy and short-chain fatty acids like cholesteryl esters can be utilized for ROS scavenging, e.g., deficiency of dietary choline or dietary lipotropes (vitamin B\(_{12}\), folate, pyridoxal, glycine, PO\(_4^{3-}\), etc.) has been reported to result in ROS production, lipid peroxidation, tissue injury, malignancy and death (Vance, 1990; Lombardi et al., 1991; Schrager and Newberne, 1993).

**Modulation of arachidonoic acid metabolism**

Two aspects of arachidonoic acid metabolism are strongly associated with carcinogenesis, and both are inhibited by antioxidants. The first is the prostaglandin synthetic pathway, and second is the prostaglandin H synthase and lipoxygenase activity. Retinoids are reported to suppress t phorbol ester mediated cyclooxygenase 2(COX2) induction in human oral epithelial cell (Mestre et al., 1997).

**Inhibition of oncogene activity**

During the course of cell proliferation in carcinogenesis, numerous oncogenes are expressed abnormally- possibly functioning as intermediates in signal transduction pathway. As for example, protein kinase.
The citrus fruit constituent D-limonene and its cogener perillyl alcohol inhibit the progression of mammary tumors induced in rats (Haag et al., 1992).

**Alternative biological pathways**

Alternative biological pathway has also been suggested for some form of cancer, as for example, stomach cancer. Gastric cancer has generally been attributed to *Helicobacter pylorii* infections, but this is also known to result from stress and trauma mediated by the cytokines. A sequence of pathological events has been elucidated: due to poor diet (low in proteins, fresh fruits and vegetables) the inflammation leads normal gastric mucosa to sparse gastric mucus, which further progresses due to *H. pylori* infection, leading to free radical formation and cytokine activation. Depletion of antioxidant resulted in nitrosamine formation and mucus barrier destruction which ultimately led to tissue injury, mutations and malignancy (Parke, 1997). Although the *H. pylorii* infection appears to be the most critical factor, this microorganism is highly resistant to antibiotic treatment.

A more successful approach appears to be to administer ascorbic acid (may be from plant sources) to inhibit nitrosation, and subsequently, with the aid of antibiotics, to eliminate the microbial overgrowth and restore the normal gastric equilibrium.

**Modulation of signal transduction**

Cells respond to signals from extracellular stimuli via a complicated network of regulated events, collectively referred to as “signal transduction pathways”. Stimulation of these pathways results in changes in transcriptional activity of genes. A recent idea about various cell-signalling pathways in ROS homeostasis is briefly discussed below. With regard to ROS homeostasis and redox regulation in cellular signalling, Ray et al. (2012) proposed an in-depth mechanism of signal transduction pathways that could be targeted for cancer therapeutics. According to Ray et al. (2012), “oxidative interphase” is the boundary between ROS and the signalling molecules they activate. The mechanism
by which ROS generation alter protein function in cellular event is stated below:

Oxidation of the redox reactive cysteine (Cys) residue forms reactive sulfenic acid (-SOH) that can form disulfide bonds with nearby cysteines (-S-S-) or undergo further oxidation to sulfinic (-SO₂H) or sulfonic acid (-SO₃H). Some time with nearby nitrogen it forms sulfenamide. These oxidative modifications result in changes in structure and functions of protein (Ross and Messens, 2011). ROS regulate several signalling pathways affecting a variety of cellular processes such as proliferation, differentiation, survival, metabolism; antioxidant and anti-inflammatory response, iron homeostasis and DNA damage response. I will touch upon a few pathways associated with oxidative stress or ROS homeostasis in this chapter, which follow several independent pathways as mentioned below, and which can serve the purpose of targeting cancer therapeutics.

**Oxidative stress or ROS-related signalling pathways for targeted cancer therapy**

**Regulation of MAPK signalling pathways by ROS**

The mitogen-activated protein kinase (MAPK) and its associated kinases are evolutionarily conserved in eukaryotes and play pivotal roles in cellular responses to a wide variety of signals elicited by growth factors, hormones, and cytokines, in addition to genotoxic and oxidative stress. ROS mediated regulation of MAPK is achieved by protein kinases and phosphatases in the MAPK signal cascade. Among the members of the MAPK cascades, apoptosis signal-regulated kinase 1 (ASK1) is an upstream regulator that functions through a number of protein cascades leading to apoptosis through phosphorylation of signal proteins. Among many ASK1 associated proteins the redox protein thioredoxin has shown to constitutively interact with ASK1 and directly inhibit its kinase activity. ASK1 plays a pivotal
role in promoting cell death under oxidative stress in mouse embryonic fibroblasts (Tobiume et al., 2001).

**Regulation of PI3K signalling pathways by ROS**

Another signalling pathway that plays a key role in cell proliferation and survival in response to growth factor, hormone, and cytokine stimulation is the phosphoinositide 3-kinase (PI3K) pathway. The PI3 K is activated by various growth factors, such as Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Nerve Growth Factor (NGF), insulin, and Vascular Endothelial Growth Factor (VEGF). Through phosphatase and tensin homology (PTEN), the PI3K triggers the reversible reaction by the stimulation of the growth factor to stop ROS production. H$_2$O$_2$ was shown to oxidize and inactivate human PTEN through disulfide bond formation between the catalytic domain Cys-124 and Cys-71 residues (Kwon et al., 2004).

**Cellular signalling mediated by Nrf 2 and Ref 1**

Redox regulation of transcription factors is significant in determining gene expression profile and cellular response to oxidative stress. Redox factor-1 (Ref-1) is a multifunctional protein that not only regulates transcription factor activity, but also mediates base excision repair. Ref-1 was shown to be up regulated by genotoxic agents and oxidants, such as bleomycin and H$_2$O$_2$, and so protected cells from DNA and oxidative damage (Ramana et al., 1998). Ref-1 was shown to be involved in the transcriptional activation of PI3K-NFE2like 2 (Nrf2) like - antioxidant response element under oxidative stress (Iwasaki et al., 2006).

**Mitochondrial oxidative stress: regulation of p66shc**

Shc adaptor protein family, encoded by the shcA locus in mammalian cells, consisting of the p66Shc is an important signalling molecule. The current understanding is that p66Shc is a pro apoptotic protein involved in ROS production in mitochondria leading to mitochondrial
damage and apoptosis under oxidative or genotoxic stress conditions, such as H$_2$O$_2$ or UV exposure. The molecular mechanism through which stress-activated p66Shc induces apoptosis has not been fully elucidated; however, p66Shc was shown to serve as a redox protein that produces H$_2$O$_2$ in mitochondria through interaction and electron transfer between p66Shc and cytochrome c, in which mutations in the redox centre of p66Shc (E132–E133 to Q132–Q133 in the CB domain,) impaired opening of the mitochondrial permeability transition pore and thus negating the pro-apoptotic function of p66Shc (Pelicci et al., 1992).

**Regulation of IRE-IRP system and iron homeostasis by ROS**

Iron is an essential element that plays crucial role in cell proliferation and metabolism by serving as a functional constituent of various enzymes including ribonucleotide reductase and cytochrome P450. However, when present in excess, free iron generates ROS via the Fenton reaction (Ghio, 2009), placing cells under deleterious oxidative stress. Therefore, tight regulation of iron homeostasis is crucial not only to maintain normal cellular function, but also to prevent iron mediated oxidative stress. Iron-responsive element (IRE) and iron regulatory protein-1 and -2 (IRP1 and IRP2) are the examples of a cascade regulatory system that not only are regulated by cellular iron status but also regulated by ROS, in which cells elicit a defence mechanism against iron toxicity and iron-catalyzed oxidative stress(Ghio, 2009).

**DNA damage response by oxidative stress or ROS**

Ataxia–telangiectasia mutated (ATM) and Ataxia–telangiectasia and Rad3-related (ATR) factors are the PI3K-like serine/threonine protein kinases activated under genotoxic stress conditions and phosphorylate various proteins involved in cell proliferation, cell death and survival, and DNA repair. These two signalling proteins were initially thought to be activated by a particular type of DNA damage, therefore serving in parallel signalling pathways; however, accumulating evidences suggest that the ATM- and
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ATR-pathways communicate and cooperate in response to DNA damage (Hurley and Bunz, 2007).

Among many, mechanisms of action of some medicinal plants and their phytochemicals that show promise in cancer prevention are outlined below:

**Some medicinal plants and plant products with chemotherapeutic applications**

*Hydrastis canadensis* (Goldenseal)

Goldenseal is a plant that grows wild in parts of the United States with its clinical use reported in skin disease, ulcers, gonorrhoea, infectious diarrhoea, eye infections, cold and other respiratory tract infections. It is occasionally used to treat cancer (Coates et al., 2005) also.

Some important derivatives of *Hydrastis canadensis* are berberine, palmatine, hydrastine, canadine (Weber et al., 2003), of which the first two components, which have been reported to be more biologically active as anti-cancer agents, will be elaborated a little.

**Berberine**

2,3- Methylene dioxy -9,10- dimethoxy protoberberine chloride is an isoquinoline alkaloid present in plants of the genera Berbis and Coptis; this alkaloid possesses antimicrobial activity against bacteria, fungi, viruses, Chlamydia and protozoan’s (Tang et al., 2009) as well as antitumor effect on many types of malignancies such as esophageal cancer (Iizuka et al., 2000), hepatoma, lung carcinoma (Mitani et al., 2001), leukaemia (Letasiova et al., 2006), uterine cancer, prostate carcinoma (Mantena et al., 2006), and gastric carcinoma (Lin et al., 2006). Berberine induces apoptosis in several human cancer cells (Hwang et al., 2002). *In vivo* study demonstrates that berberine significantly inhibits the spontaneous mediastinal lymph node metastasis (Mitani et al., 2001).

**Palmatine**

Palmatine is a close structural analogue of berberine and found in all plant families where berberine occurs,
but in a much lower amount. It bears the same tetra-cyclic structure (7, 8, 13, 13-tetrahydro-9, 10 dimethoxy-berberinium) as berberine but differs by the nature of the substitutes at positions 2, 3 on the benzo ring, being methylene dioxy for berberine and dimethoxy for palmatine (Bhadra et al., 2011). Palmatine has been used for the treatment of jaundice, dysentery, hypertension, inflammation and liver related diseases (Chang et al., 1999; Niu et al., 1996). Palmatine has significant anti-tumor activity against HL-60 leukaemia cells (Kuo et al., 1995). The anti-tumor activity is suggested to be by inhibition of reverse transcriptase and topoisomerase I and II (Sethi et al., 1983).

**Curcuma longa (turmeric or curcumin)**

Turmeric or Curcumin (food additive E100) is traditionally used as a spice and for coloring foods. Turmeric contains curcuminoids Curcumin, demethoxy curcumin and bisdemethoxycurcumin. Many health benefits such as antioxidants, antibacterial, anti inflammatory, neuroprotective and anti carcinogenic effects of curcumin are investigated. Different targets for curcumin include modulation of epigenetic mechanism, DNA methylation, histone modification, histone deacetylases, histone acetyltransferases, p53 dependent pathways, mitochondrial dysfunctioning, oxidative damages, free radical scavenging and nuclear factor kappa beta for therapeutic approaches (Esatbeyoglu, 2012).

**Chelidonium majus (greater celandine)**

*Chelidonium majus* (family: Papaveraceae) or greater celandine is an important plant in both western phytotherapy and in traditional Chinese medicine. Crude extracts of *C. majus* as well as purified compounds derived from it exhibit a broad spectrum of biological activities such as anti-inflammatory, anti-microbial, anti-tumor, analgesic, and hepato-protective (Gilca Marilena et al., 2010).

The main alkaloids of *C. majus* were found to be chelidonine, chelerythrine, sanguinarine, coptisine and berberine (Sarkozi et al., 2006).
**Lycopodium clavatum (Club mosses)**

Two hundred one lycopodium alkaloids from fifty-four species of *Lycopodium clavatum* have been reported so far; among these the major alkaloid groups are lycopodine, lycorine, fawcettimine, huperzine (Hup A), etc. which have been claimed to have specific biological action against Alzheimer’s disease, hepatotoxicity and oxidative stress (Ma and Gang et al., 2004).

**Thuja occidentalis (White ceder)**

*Thuja occidentalis* L. (cupressaceae) commonly known as eastern arborvitae is endemic to eastern North America and is grown in northern Europe as an ornamental tree in parks and churchyards (Hocking, 1997). Extracts of this plant have shown anti-viral (Beuscher et al., 1986) effect and benzo (a) pyrene protein binding inhibitory activities. Bioassay guided fractionation of this extract led to the isolation of six active constituents, namely, (+)-7-oxo-13-epi-pimara-14, 15-dien-18-oic acid; (+)-isopimaric acid; (1S,2S,3R)-(+)-isopicrodeoxypodophyllotoxin; (-)-deoxypodophyllotoxin; and (-)-deoxypodorhizone (Chang et al., 2000).

Chromatographic separation of the crude extract *Thuja occidentalis* (as homeopathic mother tincture) revealed that the Thujone-rich fraction with molecular formula of C_{10}H_{16}O and molar wt. 152 had anti-cancer potentials against A375 cells (Biswas et al., 2010).

**Gelsimium sempervirens (American yellow jasmine)**

The dried rhizomes and roots of the American yellow jasmine are indigenous to the southern United States. The ethanolic plant extract contains 45 alkaloids, many of which are toxic (Yamada, 2011). Gelsimine is the principal alkaloid and is the one most elaborately studied. Other toxic compounds are gelsemicine, 1-methoxy-and-21-oxogelsimine, 14-hydroxy gelsemicine, gelsedine, 14-hydroxy gelsedine and sempervirine. A highly fluorescent compound, possibly scopoletin is responsible for emission of blue fluorescence upon exposure to UV
light and is reported to have anti-cancer activity (Bhattacharyya et al., 2010)

**Myrica rubra (Chinese strawberry)**

*Myrica rubra*, known as Chinese bayberry is widely distributed in China, Taiwan, Korea and Japan. The bark of *M. rubra* (*Myrica Cortex*) has been used as an astringent, antidote, and anti-diarrhetic in Japanese folk medicine, and has also been applied externally for skin disease, wounds and ulcer in Chinese traditional medicine (Duke et al., 1985). Several chemical constituents such as tannins, flavonoids, and diarylheptanoids were isolated from the bark of *M. rubra* have been reported to possess hepatoprotective effects (Bao, 2005), anti-androgenic activity (Matsuda et al., 2002), and the inhibitory effects on the release of beta-hexosaminidase and on the nitric oxide (NO) production (Matsuda et al., 2002).

**Carduus marianus (Silybum marianum)**

The homeopathic tincture of *Cardus marianus* is prepared from *Silybum marianum* (Gebhardt, 2003).

Milk thistle (*Silybum marianum*) belongs to the family of Asteraceae and primarily is an indigenous plant of Mediterranean region and Southwest Europe. The seeds of milk thistle have been used for the last 2,000 years for liver disease (Vaid and Katiyar, 2010)

Silymarine is a mixture of mainly three flavonolignans, silybin(silibinin), silydianin, and silychristin (Wagner et al., 1974). Silibin is the major (70-80%) and most active biological component of silymarin. Laboratory studies suggest that there is no significant difference between silymarin and silibinin in terms of chemopreventive or biological activities conducted in several *in vitro* and *in vivo* cancer models (Singh et al., 2002; Bhatia et al., 1999).