Review

New Anticancer Agents: Recent Developments in Tumor Therapy

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Abstract. *Increasing recurrence of mammalian tumors and severe side-effects of chemotherapeutic agents reduce the clinical efficacy of a large variety of anticancer agents that are currently being used. Thus, there is always a constant need to develop alternative or synergistic anticancer drugs with minimal side-effects. One important strategy to develop effective anticancer agents is to study into anticancer agents derived from natural sources. Anticancer agents derived from plants and their derivatives have been proven to be effective for cancer prevention and therapeutics. Vinca alkaloid and their derivatives, alone and in combination with therapeutic agents, have been used for a long time for the treatment of various types of cancers. Polyphenols form one of the most important and extensively used classes of plant-derived therapeutics for cancer prevention or chemotherapy. The present review highlights a plethora of studies focused on the antineoplastic properties of plant-derived chemicals, such as Vinca alkaloid, saponins, and flavonoids.*

Despite technological and social development, cancer has become one of the most common diseases of concern and a leading cause of human suffering and death. One in 4 deaths in the United States is due to cancer. A total of 1,638,910 new cancer cases and 577,190 deaths from cancer are reported in the United States in 2012 (1). The alarming rise in incidence of new types of cancer and the public burden represents a real crisis for public health and health systems worldwide (2). Detailed analyses of pathways and mechanisms and structures of antitumor compounds have led to significant developments in the prevention and treatment of cancer. Establishment of tumor cell lines and analysis of the effect of many natural and synthetic antitumor compounds have achieved remarkable success (3). Despite their severe toxicity, chemotherapy, irradiation and immunotherapy are the gold standard approaches for the treatment of cancer worldwide. Other than these classical ways, use of natural products from plants and animals and their derivatives have produced remarkable leads for the control of cancer. Due to the toxicity of currently used therapeutics for the treatment of various types of tumors, several natural products are being tried as an alternative (4). Being less toxic, many therapeutic compounds from animal and plant sources have been extensively studied. This review focuses on newly discovered plant-derived chemicals exhibiting anticancer properties.

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Polyphenols

Fruit, vegetables and some drinks, such as tea and coffee, are particularly rich in polyphenols, and approximately 8,000 different naturally occurring polyphenols have been identified (5, 6). It is widely accepted that dietary polyphenols are beneficial for cancer prevention (7-9). Notable examples of polyphenols with anticancer effects include green tea catechins, curcumin, resveratrol and genistein. Possible mechanisms of anticancer effects of dietary polyphenols may be *via* removal of carcinogenic agents, modulation of cancer cell signalling and antioxidant enzymatic activities, and induction of apoptosis and cell cycle arrest (7, 8, 10).

Several *in vitro* studies showed that dietary polyphenols are specifically capable of affecting mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinsae (PI3K) that are involved in cancer cell proliferation (11-13). Importantly, the MAPK signalling pathway has been considered an attractive pathway for anticancer chemotherapy because of its pivotal role in regulating the growth and survival of various types of cancer cells (14). In this regard, apple procyanidins were found to inhibit cell growth, activate caspase-3 and increase MAPK levels and protein kinase C (PKC) activity in SW620 cells (a colon cancer-derived metastatic cell line) (15). Similarly, olive oil polyphenols have been shown to strongly inhibit the growth of colon adenocarcinoma cells through the inhibition of p38/cAMP response element-binding (CREB) signalling (16). However, the effect of dietary polyphenols is concentration-dependent. Low concentrations of dietary polyphenols, such as quercetin, green tea polyphenols and epigallocatechin gallate (EGCG), can activate MAPK pathways, leading to the expression of survival genes *e.g. c-fos* and *c-jun,* (17) whereas higher concentrations of quercetin and EGCG can activate the caspase pathway that leads to apoptosis (18-20).

Several studies have shown that the anticancer ability of some dietary polypehnols such as quercetin, luteolin, genistein, apigenein, and resveratrol, is attributable to the induction of apoptosis of various cancer cells as well as in animal models (6-9, 21-23). EGCG also exhibited an apoptosis-inducing effect in numerous cell lines by increasing expression of fatty acid synthase (FAS) and caspases, as well as by the inhibition of apoptosis-suppressing proteins, B-cell lymphoma (BCL)-2, BCL- extra large (BCL-xL) and (BH3) interacting domain death agonist (24-27). Interestingly, ellagic acid and quercetin synergistically induce apoptosis in diverse cancer cell lines (28). Moreover, black tea extract (T5550) enriched in theaflavins inhibited the chymotrypsin-like (CT) activity of the proteasome and proliferation of human multiple myeloma cells in a dose-dependent manner. An isolated theaflavin (TF-1) was also found to bind to, and inhibit purified 20S proteasome, accompanied by suppression of tumor cell proliferation (29).

The incidence of gastrointestinal cancer has risen rapidly over the past three decades. Some early studies correlated tea consumption with stomach cancer (30). More recent studies on green leaf tea showed that its consumption reduced the risk of stomach cancer by 81% and esophageal cancer by 39% in alcoholics. It also reduced the incidence of stomach cancer by 16% and esophageal cancer by 31% among cigarette smokers (31). In a recent study in China, green tea was found to reduce the risk of esophageal and gastric cancer (32). A population-based cohort study of 74,942 Chinese women suggested that regular intake of green tea may delay the onset of breast cancer. Moreover, out of women under 50 years of age, those who consumed tea were 37% less likely to develop breast cancer compared to women who did not. Wu *et al*. reported the correlation between catechol-o-methyl transferase (*COMT*) allele, intake of tea and occurrences of cancer. They observed that women who possess at least one low-activity *COMT* allele had a reduction in breast cancer risk with intake of tea, but in the case of these homozygous for the high-activity *COMT* allele, there was no effect of drinking tea on breast cancer onset (33). An interesting study showed that green tea reduced malignancy in prostate cancer among green tea-treated individuals as compared to untreated ones. No significant side-effects or adverse effects were documented (34).

Saponins

Saponins, a class of bioactive compounds naturally present in many plants, are a major family of secondary metabolites containing a sugar moiety glycosidically linked to a hydrophobic aglycone (sapogenin). Saponins have emerged as natural detergents and foaming agents, with cardiac, immunostimulatory, and anticancer activity, and other health-promoting functions (35, 36). Saponins allow plants to cope with environmental stress such as storing and conserving water, resisting predators, and surviving severe weather conditions. Saponins have detergent and surfactant properties because they contain both water-soluble (the sugar moiety) and fat-soluble (sapogenin) subunits. Plant sources of saponins include yucca, Christmas rose (*Helleborus niger*), horse chestnuts (*Aesculus hipppcastanum*), asparagus fern (*Asparagus officinalis*), daisies (*Bellis perennis*), chickpeas, soybeans and alfalfa (37). The cholesterol-binding attribute of saponins is related to their ability to inhibit the growth, or kill cancer cells that have more cholesterol-type compounds in their membranes than normal cells. The polarity, hydrophobicity, and nature of the reactive groups of saponins are important determinants of their biological properties (38).

The most potent compounds in soybean were shown to be the aglycones soya sapogenol A and B, inducing almost complete suppression of cell growth. Saponins from soybean suppressed the growth of HT-29 colon cancer cells (37). Soybean extracts also exhibited synergistic antiproliferative activity against an ovarian tumor cell line (OVCA 433) (37, 39). Several glycosides (naringin, rutin, and baicalin) of soybean origin exhibited anticancer activity (40). In humans, rutin attaches to iron ion (Fe^{2+}), preventing it from binding to hydrogen peroxide, which would otherwise create highly reactive free radicals that can damage cells (41). Baicalin had a cytotoxic effect on leukemia-derived T-cells (42). The aglycone sapogenol exhibited antiproliferative activity against MCF-7 breast cancer cells (43).

Saponins isolated from *Balanites aegyptica* exhibited cytostatic activity against P-388 lymphocytic leukemia-cultured cells (44). A mixture of the steroidal saponins

balanitin-6 and balanitin-7 (Bal 6/7), isolated from *B. aegyptiaca* kernels, demonstrated appreciable anticancer effects against A549 non-small cell lung cancer and U373 glioblastoma cell lines. Moreover, Bal 6/7 increased the survival time of mice bearing murine L1210 leukemia to the same extent as that reported for vincristine. These observations suggest that Bal 6/7 could be used to generate novel semisynthetic derivatives with potentially improved *in vitro* and *in vivo* anticancer activity and reduced *in vivo* toxicity, thus markedly improving the therapeutic ratio (45). Saponins from *Agave schottii*, a Sonora Desert xerophyte plant of the Agavaceae family, were effective inhibitors of a Walker carcinoma 256 tumor system (46). Moreover, steroid saponins derived from yucca (*Yucca schidigera*), a xerophyte also belonging to the Agavaceae family, display carcinostatic and mutagenesis inhibitory effects, and are thus capable of inhibiting tumors (47-49). Another desert plant Quillaja (*Quillaja saponaria*), a Quillajaceae family drought-resistant evergreen tree native to warm-temperate central Chile, is used in folk medicine by the Andean people (48, 50). The antiproliferative activity and mode of action of spirostane (SAP-1016 and SAP-884) and furostane (KE-1046 and KE-1064) saponins, isolated from *Balanites aegyptiaca* were investigated. The compounds SAP-1016 (3β-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside) exhibited potent antiproliferative activity against MCF-7 human breast cancer cells and HT-29 human colon cancer cells, as compared to a well-known anticancer agent, cisplatin (51). A recently patented anticancer preparation contains extracts from Schisandra, Trichosanthes, yucca plants and glycine and claims to induce apoptosis or cell-cycle stasis and inhibit angiogenesis or tumor cell metastasis, and to be useful for the treatment of cancer and cell proliferation disorders (52).

Vinca Alkaloids

Vinca alkaloid, derived from *Catharanthus roseus*, is one of the most extensively studied classes of antineoplastic agents. Dimeric alkaloids from *C. roseus*, in combination with chemotherapy, have been widely used for treating solid tumours. Currently available Vinca alkaloids, vinblastine, vincristine, vindesine and vinorelbine are used in daily clinical practice (53). Among the numerous derivatives synthesized, only vindesine and vinorelbine, semisynthetic analogues of vinblastine, are in clinical use. More recently, a new Vinca alkaloid, known as vinflunine, has been developed (54).

The antitumor properties of Vinca alkaloids derive from their interaction with tubulin, the major component of microtubules in mitotic spindles. These drugs interfere with the dynamics and assembly of microtubules resulting in cell division arrest in metaphase (55-57). Vinorelbine and vinflunine, the second generation of Vinca alkaloids, suppress the rate and extent of microtubule growth and enlargement, affecting mitotic spindle functions, leading to modifications of cell-cycle progression and cell killing (58). Vinflunine is a specific inhibitor of tubulin that prevents microtubule assembly during mitosis (59) and induces apoptosis (60). The molecular mechanisms of cell killing by vinflunine also include a series of events leading to apoptotic cell death (59, 61). Furthermore, several studies have proposed a role for BCL-2 phosphorylation in the apoptotic response of tumour cells to microtubule-damaging agents (31). The apoptosis mechanisms induced by vinflunine involve caspases 3/7 and c-Jun N-terminal kinase 1 activation, but do not require Bcl-2 phosphorylation. Vinflunine also exhibited antivascular effects restricting the growth of tumor by shutting down the tumour vasculature (62). Antivascular effects could be due to vascular-disrupting effects, endothelial cell morphological changes, newly formed deficient capillary-like structures, inhibition of endothelial cell motility and proliferation, and prevention of endothelial cells from correctly aligning to form capillary-like structures. Vinflunine was also inhibitory towards fibroblast growth factor (bFGF)-induced angiogenesis (63, 64). Vinflunine exhibited *in vitro* antiproliferative effects in several murine and human tumour cell lines (59), and also *in vivo* anti-tumour activities against subcutaneous tumour xenografts (60). Vinflunine also demonstrated greater superiority over vinorelbine in a number of studies (65). In addition, lower doses of VFL (*i.e.*, 16-fold lower than the maximal therapeutic dose), also reduce the number of experimental liver metastases induced by colon cancer cells (64). Recent clinical trials have established the feasibility and promising efficacy of vinflunine as a second-line therapy in patients with advanced bladder cancer who have failed a prior platinum-containing regimen (66). It was approved by the European Medicines Agency in September 2009 for the treatment of adult patients with advanced or metastatic transitional cell carcinoma of the urothelial tract (67).

Moreover, the combination of vinflunine with gemcitabine was shown to be active in patients with non-small cell lung cancer (NSCLC) (68). Patients with metastatic breast cancer (MBC) whose anthracyclines and taxanes therapy failed, experienced promising antitumour activity when treated with the combination of vinflunine and capecitabine and this combination was found to be safe with minimal side-effects. Further clinical development of this combination is warranted (69).

Miscellaneous Agents for Cancer Prevention

Some rare compounds have also been exploited for their anticancer property. Nordihydroguaiaretic acid, a naturally occurring lignin from creosote bush (*Larrea divaricata Cav.* or *Corillea tridentate*), and its synthetic analogues are

potentially useful in treating cancer. Remarkably, terameprocol, a tetra-*O*-methyl derivative of nordihydroguaiaretic acid, is in phase I/II clinical trials as an anticancer agent (70). Thymol, piperitone, and methyleugenol, essential oils from the root of *Anemopsis californica* inhibited the growth of human endometrial cancer cell-line AN3CA and of the cervical cancer cell line HeLa (71). Iridoids, bioactive compounds in the roots and rhizomes of plants belonging to the genus Valeriana (*Valerianaceae*), are known to be inhibitors of cell migration (72). *Ammopiptanthus mongolicus* and an *Ammompiptanthus mongolicus* lipid, traditionally used in China have been shown to inhibit liver cancer (73). Ethyl acetate fractionated extracts of Calligonum comosum (Polygonaceae) demonstrated anticancer properties (74). Two compounds, terrequinone A and terrefuranose derived from rhizosphere fungi, displayed selective cytotoxicity against cancer cell lines compared with the normal fibroblast cells (75). *Pituranthos tortuosus* extracts have been reported to demonstrate antiproliferative and apoptotic properties using leukemia cell lines (76). Terpinen-4-ol, sabinene, α-terpinene, and β-myrcene isolated from *P. tortuosus*, exhibited significant cytotoxicity towards against human cancer cell lines, namely, human hepatocellular liver carcinoma cell line HepG2, colon cancer cell line HCT116, and breast cancer cell line MCF7 (77). A cycloartane-type triterpenoid, an aliphatic alcohol glycoside, eudesmane-type sesquiterpenoid, and a guaiane-type sesquiterpenoid, isolated from the resinous exudates of *Conmmiphora opobalsamum*, showed moderate antiproliferative effects on human prostate cancer cell lines and inhibited the expression of androgen receptor in LNCaP cells (78). It was found that extracts derived from *Varthemia iphionoides*, exhibited cytotoxicity against leukemia cell proliferation (79). Polyphenols and sterols of virgin argan oil exhibited dose-dependent cytotoxic effects and antiproliferative actions on three human prostatic cell lines (DU145, LNCaP, and PC-3) (80, 81). *Teucrium polium* plant extract inhibited cell proliferation and induced cell cycle arrest and reduction of the G_0-G_1 phase, suggesting therapeutic potential against metastatic disease (82).

Many cytotoxic compounds have been isolated from tunicates, also known as urochordates, which belong to the subphylum protochordate (83). Compounds derived from the Didemnidae family are structurally unique, and include alkaloids and various peptides. For example, fascaplysin is an alkaloid isolated from two *Didemnum tunicates* and four other distinct types of sponges (84). Didemnid ascidians hosting the symbionts *Prochloron sp*. have yielded distinctively related cyclic peptides with cytotoxic activity (85). Some other antitumor compounds, isolated from *Ascidian lissoclinum* are haterumaimides F–I, J–K and N–Q, showing different levels of cytotoxic potential against P388 leukemia cells (86).

Haterumaimide J and K obtained from *Lissoclinum* sp. exhibited cytotoxicity against murine leukemia P388 cells (87). Dichlorolissoclimide, chlorolissoclimide from *Lissoclinum* sp. showed an antiproliferative effect due to blockage of G_1 phase cells against the non-small cell bronchopulmonary carcinoma line NSCLC-N6 (88). Cyclopentenones from *Lissoclinum* sp. also showed significant cytotoxicity towards human colon carcinoma HCT116, epidermal cancer line A431 and the human alveolar basal epithelial adenocarcinoma line A549 (89). Lissoclibadin and lissoclinotoxin, obtained from *Lissoclinum cf. badium* showed a wide range of inhibitory effects against the human colon cancer lines DLD-1 and HCT116, the breast cancer line MDA-MB-231, the renal cancer line ACHN and the NSCLC line NCI-H460 (90).

Tuberatolide A, tuberatolide B and 2'-epi-tuberatolideB, obtained from *Botryllus tuberatus* inhibited the chenodeoxycholic acid -activated human farnesoid X receptor (hFXR) without significant effect on steroid receptors (91). Some of the compounds obtained from *Sidnyum turbinatum* showed *in vitro* antiproliferative activity against the mice fibrosarcoma cell line (WEHI164) (92). Moreover, haouamine A and haouamine B from *Aplidium haouarianum* exhibit selective cytotoxic activity towards the HT-29 human colon carcinoma cell line (93).

Conclusion

Pharmacological activities associated with natural products have been recognized since the beginning of mankind; however only limited numbers of medicinal plants and other natural products have been scientifically evaluated so far. Many plant products and their chemical derivatives have been used in therapeutics of serious diseases such as cancer. Although a growing body of plant-derived products have been reported to prevent tumor growth the exact underlying molecular mechanisms of most of these agents remains to be elucidated, particularly with respect to kinetic approach towards active site of the target enzymes. Understanding the cross talk between these plant products and proteins in various signalling pathways would be a step forward in development of anticancer drugs. Different biochemical and biophysical approaches such as co-crystallization and three-dimensional structure determinations could be adopted for further dissecting their molecular mechanisms. In addition, *in silico* approaches like molecular docking could also be of significant importance in understanding the interaction of these products in different signalling pathways which can be further validated by various *in vitro* and *in vivo* studies. There is a growing demand for testing these products in clinical trials, which is possible only after gaining the insight into the molecular interaction of these plant-derived chemicals with different signalling molecules.

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