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Review

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Potentials of plant products as anticancer agents

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Abstract

Cancer has remained a major health concern. Advances in existing therapeutic modalities have failed to provide cure, prevent a relapse and are always accompanied by serious and often times debilitating side effects. Extensive screening of plants for anticancer activity has produced some encouraging and impressive results. More than 40,000 plant species have reportedly been screened for anticancer effects. Some of such plants include - *Podophyllum hexandrum, Podophyllum petatum, Catharanthus rosea, Taxus brevifolia, Taxus baccata, Camptotheca accuminata* etc. Anticancer principles from plants include among others, alkaloids of *Vinca rosea* (Vinblastine and Vincristine), *Ocheosia elliptica* (ellipticine and α - methoxyellipticine), toxin of *Ipomea batatas* and ricin from *Ricinus communis* . Mechanisms proposed to underlie the anticancer effect of these plants include mitotic arrest in the S, G, and metaphase phases, inhibition of normal mitotic spindle formation, inhibition of microtubule depolymerization and topoisomerase inhibition. Some of these plants/principles are already in use while some are in the clinical trial stages of drug development. Active plant principle can also provide templates upon which synthetic and semi-synthetic derivatives can be produced such as the active analogue of Camptothecin: 9-aminocamptothecin, topotecan and irinotecan. The success recorded from the relatively small number of plant species so far screened out of an estimated plant population of half a million worldwide indicate the enormous therapeutic potential inherent in natural endowments of plant origin. With more than 90% of the plant population still unexploited, plants definitely hold hope for the discovery of potent anticancer agents with minimal side effects/toxicity profile and capable of preventing a relapse.

Keywords: Plant products, anticancer, podophyllum, vinca alkaloids, taxol, camptothecin, curcumin

1. Introduction

Cancer is essentially a problem of abnormal cell growth. Under the influence of chemicals in the environment, radiation or viruses, the DNA in normal cells may be transformed, possibly by a single alteration or substitution of one of the constituent purine or pyrimidine bases, in such a way that the normal control mechanisms, which restrict cell proliferation are removed.

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Consequently, the cell may reproduce uncontrollably, invade surrounding tissues, and eventually spread to different parts of the body to form secondary growth or metastasis. This makes cancer a particularly difficult disease to combat [1-3].

Various therapeutic modalities have been employed in the fight against cancer [4-6]. These include: alkylating agents, [5-7] anti-metabolites [4, 8], natural products [9,10] radiomimetic drugs or ionizing radiation, [11-16] hormones and antagonists [17], biological response modifiers [3, 18-22], surgery, [23, 24] and miscellaneous agents [4, 24-28]. However, none of these agents (single or in combination) has produced satisfactory anticancer effect without relapse [29] and most times, their therapeutic activity is accompanied by debilitating side effects [30-32].

The increased epidemics of Acquired Immunodeficiency Syndrome-related cancer [33-36] demand that the search for more anticancer agents be pursued with renewed vigour. In this invigorated effort, a vast amount of synthetic work has given relatively small improvement over the prototype drugs.

Consequently, there exists a need for new prototypes and templates to be used in the design of potential chemotherapeutic agents, and plant products are providing such templates. Studies of tumour-inhibiting compounds of plant origin are yielding an impressive array of novel structures. Many of these structures are extremely complex and it is most unlikely that such compounds would have been synthesized in empirical approach to new drugs [10, 37, 38].

In addition, the cost of existing antineoplastic drugs brings the issue of availability and affordability at conflict. The need to provide anticancer drugs that have therapeutic efficacy across wide variety of tumours without relapse, reduced side effect profile, cost-effective and are easily available, prompted recourse to plant products for a template or prototype.

2. Plant products as anticancer agents

Plant materials have been used in the treatment of malignant diseases for centuries [32 - 39], as of the beginning of 1970s literature describing plants used against cancer lists over 1400 genera. An intensive survey of plants, microorganisms and marine animals for antitumour activity began in the late 1950s with the programme initiated by the United States Cancer Institute (NCI). From the beginning of the programme to early 1980s, 114,000 plant samples representing 40,000 species have been tested and more are still being tested till date. Some of the promising candidates are discussed below.

2.1. Podophyllum

Podophyllum (Podophyllum rhizome, May-apple root, Wild Mandrake) was used over 2000 years ago by the ancient Chinese as an antitumour drug, and resins from the roots of the plant, *Podophyllum hexandrum* (syn. emodi), and the related American species, the May-apple (*P. petatum*) have yielded a number of ligands and their glycosides were reported to have antitumour activity [10, 37].

The underground stem of *P. petatum* was used years ago by Indians to treat cancer [40]. The resin from this species was recommended in American Materia Medica more than 100 years ago for the treatment of cancerous tumours, polyps and unhealthy granulomas [41]. Podophyllum resin, or podophyllin, was used by Physicians in Mississipi and Missouri as early as 1877 and by urologists in Louisiana for the treatment of veneral warts (*Condyloma acuminata*) [42].

The satisfactory use of these drugs has been complicated by toxicity [43], a disadvantage that has been reduced by the use of derivatives, such

as epipodophyllotoxin [44]. Two semisynthetic glycosides of the active principle, podophyllotoxin, have been developed. These derivatives (epipodophyllotoxins) referred to as etoposide (VP-16-213) and teniposide (Vm-26) showed significant therapeutic activity in several neoplasms [4].

Mechanism of action: It appears that etoposide and teniposide are similar in action and in the spectrum of human tumours affected [45, 46]. Unlike podophyllotoxin, they do not cause mitotic arrest by binding to microtubule. Rather, at low concentration, they block cells at the G_2 interface of the cell cycle and, at higher concentration, they cause G_2 arrest. The greatest lethality is seen in the S and G_2 phases [4].

Growing evidence indicates that the epipodophyllotoxins stimulate DNA topoisomerase II to cleave DNA [45, 47]. Resistant cells demonstrate either amplification of the multi-drug-resistant P-170 glycoprotein that promote drug efflux [48, 49], alteration of topoisomerase II [48, 49] or increase detoxification by glutathione-dependent enzyme [45].

Therapeutic uses: The semi-synthetic podophyllotoxin derivatives, etoposide and tenoposide, are the most active drugs used as single agent for the treatment of small cell lung cancer and have shown considerable activity against testicular and malignant lymphoma [45, 47]. Clinical trials have also shown these drugs to be effective against CNS tumour, [45] and Kaposis sarcoma, a tumour associated with AIDS.

They are also active in acute nonlymphocytic leukemia, and carcinoma of the breast [4]. Tenoposide is available for investigational use, and it has orphan drug status for treatment of refractory acute lymphoblastic leukemia in children [4].

The strand-breaking activity of etoposide/ tenoposide has been implicated as a mechanism of cytotoxicity and a positive correlation has been found between etoposide toxicity and DNA breaks in several tumour cell lines [51, 52]. The most frequently encountered toxicity is myelosuppression (leucopenia) and this is dose-dependent [53, 54]. Alopecia is common but reversible [4, 53]. Other common side effects include nausea, vomiting and diarrhoea [4].

2.2. Vinca alkaloids

The beneficial properties of the periwinkle plant (*Vinca rosea*, Linn.), a specie of myrtle, have been described in medicinal folklore for many years [55, 56]. While exploring claims that the extract might have beneficial effect in diabetes mellitus, granulocytopenia and bone marrow suppression were observed in rats [57]. Purification and fractionation of these extracts yielded four active dimeric alkaloids; two of which, vinblastine (Velban ®) and vincristine (Oncovin®) are important clinical agents [47, 55].

Mechanism of action: The vinca alkaloids interfere with microtubule assembly, causing metaphase arrest in a cell-cycle-specific manner [58]. In the absence of intact mitotic spindle, the chromosome may disperse throughout the cytoplasm (exploded mitosis) or may occur in unusual grouping, such as balls or stars. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death [4, 9].

Therapeutic uses: Vincristine has a spectrum of clinical activity that is similar to that of vinblastine but there are some notable differences. Vincristine is clinically more important than vinblastine and is especially useful in the treatment of childhood leukemia; it is also the main component of several highly effective combination regimens [57]. Vincristine is more useful than vinblastine in

lymphocytic leukemia. Vincristine and vinblastine are the most commonly used antineoplastic drugs in AIDS- associated Kaposis sarcoma [59, 60].

They are also used in HIV-associated Hodgkin's sarcoma [60]. Beneficial response has been reported in patients with a variety of other neoplasms, particularly Wilm's tumour, neuroblastoma, brain tumour and carcinoma of the breast, bladder and the male and female reproductive systems [61]. Vinblastine is mainly used in the treatment of Hodgkin's disease, a cancer affecting the lymph glands, spleen and the liver.

Vinblastine has been structurally modified to yield desacetylvinblastine amide (Vindesine), which has been introduced for the treatment of acute lymphoid leukemia in children. Vinorelbine is a semi-synthetic compound derived from the vinca alkaloid series. This new agent has shown activity when given as first line chemotherapy in patients with recurrent head and neck cancer [62, 63].

The clinical toxicity of vincristine is mostly neurological with little myelosuppressive effect [58]. Several types of neurological toxicities have been observed and include peripheral neuropathy, [64], poly neuropathy [65], numbness and tingling of the extremities followed by weakness, loss of reflexes, foot-drop, ataxia, muscular cramps and neuritic pains [4]. Vinblastine is more myelosuppressive than vincristine, but causes less neurotoxicity while both of them cause alopecia and constipation [58].

Although less common than with vinblastine, leucopenia may occur with vincristine and thrombocytopenia, anemia, polyuria, dysuria, fever and gastrointestinal systems disorder have been reported occasionally. The myelosuppressive effect of vinblastine could be ameliorated by dose reduction or the use of cytokine [60]. The toxicities of vincristine and vinblastine may also be ameliorated by giving the drugs weekly on an alternating basis [66].

2.3. Paclitaxel (taxol®)

Taxol is the first of a new group of drugs termed the taxanes. The activity of taxol (paclitaxel) was first reported in the late 1960s when a crude extracts of the bark from pacific yew, *Taxus brevifolia* (Fam. Taxaceae), was evaluated for cytotoxic activity as part of the large natural product screening program of the U.S. National Cancer Institute (NCI) [67, 68].

Mechanism of action: Taxol is a diterpenoid plant product that exhibits significant anti-tumour activity against various malignant cells such as ovarian [69, 70], breast and lung cancer cells [71], malignant melanoma [92], as well as leukemias [76, 77]. Interest in this compound stems not only from its clinical activity against poorly responsive solid tumours, but also from its unique mechanism of action [75, 76].

Unlike other antimitotic compounds such as vincristine and colchicine that inhibit tubulin polymerization and microtubule formation, taxol enhances tubulin polymerization, stabilizes microtubule, and prevents microtubule depolymerization induced by calcium or low temperature [77, 78]. The unusual stability of microtubules in taxol-treated cells leads to mitotic block, resulting in inhibition of cell division [79, 80].

There are two evidences in support of this model of action; (1) most taxol-treated cells are arrested in G_2/M phase [81, 82], (2) cells in mitotic phase are found to be more sensitive to taxol than those in interphase [83, 84]. In addition, taxol induces nuclear fragmentation, a hallmark of apoptosis, in many cell lines [85-87]. Apoptosis occurs not only upon treatment with

high concentrations of taxol, but also upon prolonged treatment with low concentration of this drug [80,88]. However, the detailed mechanism of its cytotoxicity remains elusive.

Therapeutic uses: Taxol has activity against carcinoma of the ovary where a response rate of 30 - 36% has been reported [89, 90]. It has been found effective in HIV - associated Kaposis sarcoma [91] as well as in malignant melanoma, breast cancer and non-small cell lung carcinoma [68]. The dose-limiting toxicities of taxol are reported to include myelosuppression, neurotoxicity and cardiotoxicity. Transient arthralgia and myalgia, alopecia, mild nausea, vomiting and diarrhoea have been experienced [58, 92].

In addition, patients experienced hypersensitivity reactions. Routine premedication with a corticosteroid, an antihistamine and a histamine H_2- receptor antagonist is recommended to prevent severe hypersensitivity reaction [58, 92, 93].

Docetaxel is a semi-synthetic product of European yew, *Taxus baccata* [94]. The cytotoxic activity of docetaxel and other taxoids such as paclitaxol is believed to be largely related to their ability to enhance microtubules by preventing their depolymerization. Microtubules are formed when α and β tubulin subunits copolymerize. Normally, a dynamic equilibrium exists between tubulin and the assembled microtubule [96].

Although the mechanism of action of docetaxol and paclitaxel are similar, there appear to be some differences. The two agents promoted formation of structurally distinct microtubule [97, 98] and were active during different phases of cell cycle [99]. Docetaxel was reported to be preferentially active during S phase and had only partial activity during the G_2/M phase whereas paclitaxel had its greatest activity during the late G_2/M phase [99]. Docetaxel has been investigated for use in patients with different types of solid tumour [73].

It has an *in vitro* activity against a wide range of tumour cell types including breast, ovarian, prostatic, lung, colorectal, gastric, melanoma and renal cancer cell lines and/or freshly explanted tumour cells [100 - 106]. Paclitaxel and docetaxel are both currently manufactured via semisynthetic process using the yew needles, a renewable resource [107].

Adverse effects of docetaxel from phase I and II trials have been reviewed [108]. In clinical trials, docetaxel has shown similar activity to paclitaxel, but with considerable greater toxicity, notably peripheral oedema, pleural effusions, skin and nail toxicities and profound lethargy [109]. Although such effects can be delayed and reduced by pretreatment with corticosteroids, they remain significant adverse effects, which limit the dose and duration of docetaxel treatment [93].

Other taxanes: The needles of *Taxus baccata* L. contain an impressive array of taxane diterpenoids. However, the content of the antitumour taxol in the needles of these species is generally low. However, the development of Taxus varieties with high content of the taxanes is of considerable practical importance [110] for semisynthetic purpose of clinically more important taxol or paclitaxel.

2.4. Topoisomerases inhibitors

DNA topoisomerases (topo I and II) are nuclear enzymes responsible for controlling, maintaining and modifying structures or topology of DNA during replication and translation of genetic materials. In order to perform those functions, topoisomerases induce transient cuts in one or both strands of the DNA, allowing strands to pass through the nick, and then rejoining the nicked strand to DNA.

During this normal function of topoisomerases, a covalent linkage is formed between topoisomerase and DNA called trappable or cleavable complexes. Topo-active anticancer drugs stimulate and stabilize this complex, causing strand scission and inhibition of the DNA function. Because different classes of drugs bind DNA with sequence specificity, different agents induce site-specific DNA damage [111 - 115]. There are many common and overlapping functions of topo I and topo II in maintaining DNA topology. Inhibition of one form of topo results in an increase in the activity of the other topo.

However, there are some major differences. Whereas the activity of topo II is highest in log phase and fast growing tumours, topo I is not a cell-cycle specific enzyme; topo I only induces single strand breaks whereas topo II induces both single and double-strand breaks; and topo I function is independent of ATP, while ATP is required for topo II function [116]. Plant extracts from *Camptotheca accuminata* was described to have significant antitumour activity against leukemia and solid tumours in the 1960s [117].

Subsequent work led to the structural and chemical identification of its active fraction camptothecin - which showed activity against a wide variety of tumour cell lines *in vitro* [117, 118]. Clinical trials with camptothecin indicated a lack of significant activity as well as excessive toxicity, especially haemorrhagic cystitis and myelosuppression.

Consequently, structural modification led to the identification of several active analogues of camptothecin, some of which - NS-603071 (9-aminocomptothecin, 9-AC), topotecan and irinotecan (CPT - 11) - are currently undergoing clinical trials [119, 121]. Camptothecin and its derivatives have been identified to have topo I inhibitory activity.

This enzyme catalyzes the cleavage and release of supercoiled DNA, an essential step in DNA replication and transcription [116]. Binding of camptothecin to topoisomerase I prevents its release from DNA. This action inhibits DNA and RNA synthesis in tumour cells and induce protein-associated single [122] and double [123] - stranded DNA breaks which can lead to cell death. The cytotoxic activity is time-dependent [124].

Topoisomerase 1 inhibitors are of great clinical interest because of their unique mechanism of action, high activity in preclinical tumour models and high expression of the enzyme in various human tumour types [125-129]. NSC - 603071 is a more water-soluble analogue of camptothecin and has topoisomerase 1 as the main cellular target [110]. NSC - 603071 was shown to be active against p-170-positive tumours, indicating no cross - resistance to multi - drug resistant Mdv 1- positive cells [116, 130].

Other camptothecin derivatives reported to have antitumour activity include topotecan [131-133] and irinotecan [134-137]. Liriodenine, a cytotoxic oxoaporphine alkaloid, has been isolated from plant species of many genera such as *Cananga odorata* [138, 139]. Its synthesis has also been reported [140]. Liriodenine has a remarkable range of biological activity.

It is cytotoxic to human cancer cells [141, 142] and active against Gram-positive bacteria, yeast and filamentous fungi [143, 144]. Liriodenine is a mutagen [145] and a clastogen, which causes chromosomal aberrations at low doses [146].

Recently, this cytotoxic and neoplastic drug has been found to be a potent catalytic inhibitor of topoisomerase II both *in vivo* and *in vitro* [139]. Liriodenine rapidly crosses cell membranes and efficiently blocks the topoisomerase II dependent step in DNA

replication [139]. Topoisomerase II has been shown to be required for the separation of newly replicated cellular chromosomes in eucaryotic cells [147]. Failure to separate the chromosomes results in cell death.

Since purely catalytic inhibitors of topoisomerase II can have significant anticancer activity, it is likely that catalytic inhibition of topoisomerase II contributes to the anticancer activity of liriodenine [139].

It is also suggested that liriodenine may be a weak topoisomerase II poison [139]. Inhibition of topoisomerase II is also the likely basis of liriodenine's clastogenic and mutagenic activity [142]. Topoisomerase II poisons are well known for causing chromosome damage including illegitimate recombination [148, 149], deletion [150], sister chromatid exchange [151] and translocations [152, 153]. Topoisomerase 11 poisons have been reported to be mutagenic [154, 155].

2.5. Curcumin

Curcumin (diferuoylmethane), the yellow pigment in the rhizome of tumeric (*Curcuma longa*), an ingredient of curry spice, is known to exhibit a variety of pharmacological effects, including antitumour, antiinflammatory, and antiinfectious activities [156].

The anti-carcinogenic properties of curcumin in animals have been demonstrated by its inhibition of both tumour initiation induced by benz [α] pyrene and [7, 12] - dimethylbenz [α] anthracene and tumour promotion of phorbol esters [157, 158]. Recent results have indicated that dietary administration of curcumin significantly inhibits forestomach, duodenal, colon and tongue carcinogenesis in mice and rats [159-161]. Although the exact mechanism underlying these effects of curcumin remains to be elucidated, the antioxidant properties of this compound are likely to be involved [162].

Several independent studies have shown that curcumin inhibits lipid peroxidation [163, 164] and free radical generation1 [165, 166] as well as possessing scavenging properties [167], thus serving to protect various cellular constituents, including DNA from oxidative injury [168]. Curcumin inhibits cell proliferation [156].

This could be explained by its capacity to inhibit diverse protein kinases, such as protein kinase C [169] and phosphorylase kinase [170]. The ability of curcumin to inhibit the growth of mouse 3T3 cells has been correlated with a decrease in epidermal growth factor receptor phosphorylation [171].

This pigment has also been shown to inhibit expression of several proto-oncogens [172, 173]. The activity of the AP-1 transcription factor is suppressed by curcumin [174-176]. Studies showed that curcumin not only inhibits proliferation of rat thrombocytes and human Jurkat cells (neoplastic lymphoid cells) but also apoptosis of these cells [156]. These two inhibitory activities of curcumin correlate with the suppression of AP-1 activity [156].

Among others, the possible mechanism of cell proliferation and cell death inhibition by curcumin lies on the fact that the pigment possesses strong antioxidant and reactive oxygen species (ROS) scavenging properties [161]. Hence, it may be expected to exert its inhibitory activity by influencing the cellular redox state, ROS detoxification, and inhibition of ROS generation. ROS involvement in cell death and cell proliferation in particular has strong experimental support [177, 178].

On the other hand, AP-1 activation is usually observed under antioxidant, but not under pro-oxidant conditions [179]. Thus some additional investigations are required to clarify the role of curcumin as anti-oxidant and ROS scavenger in apoptosis.

2.6. Other putative antitumour agents from plants

Several other plant products have also shown interesting anti-tumourigenic activity. Ellipticine, a pyridocarbazole alkaloid, and 9-methoxyellipticine, both derived from *Ochrosis elliptica* (Apocynaceae), have shown potent inhibitory activity against several malignant disorders but preclinical studies indicated a number of side-effcts [180-181].

Through its potent antiangiogenic activity, AGM 1470, the synthetic analogue of fumagillin (isolated from *Aspergillus fumigatus*), exhibits marked antitumour activity without the side effects of fumagillin [182-185]. The diterpenes triptolide and tripdiolide isolated from *Ttipterygium wilfordii* are potent antileukemic agents that contains reactive triepoxide [180]. Extracts from betel leaf, *Piper betel*. L. (Piperaceae) have been shown to reduce the number of papillomas in animals [186].

Extracts from *Cyclea peltate* and *C. barbata* (Menispermaceae) have been used in cancer chemotherapy [187]. The germacranolide, elephantopin from *Elephantopus elatus* is among four of the many sesquiterpene lactones tested that showed *in-vivo* antitumour activity [180].

Favourable results have been reported in clinical studies using alkaloidal fraction of *Cephalotaxus harringtonia*, and there is hope that homoharringtonine in particular may be active in patients with solid tumour or leukemia [180].

In Moroccan traditional medicine, seeds of *Peganum harmala* have been used for the empiric treatment of cancer and recently, alkaloid fraction of the methanolic seed extract has been shown to be active against cancer cell lines in rats [188]. *Tinospora cordifolia* stem extract has been demonstrated to reduce solid tumour growth and synergistically acted with cyclophosphamide in reducing animal tumours; this action is suggested to be through the stimulation of effector cells that retard/destroy the tumour cells [188].

Psoralen from *Psoralea corylifolia* has been shown to be active against cutaneous T cell lymphoma [189] and cultured mucoepidermoid carcinoma cells of MEC-1 cell line [190]. The seed extract was found to stimulate natural killer cell activity, antibody-dependent cellular cytotoxicity, antibody-forming cells and the antibody complement-mediated cytotoxicity during tumour development [191]. The seed is used in ancient Hindu remedy for leucoderma and vitiligo [192].

Plant products have contributed a lot in the development of cancer chemotherapy and many cancer patients rely on these products for relief. The potentials of plant in providing a lead anticancer structure is inestimable. A vast array of plant products are still untapped and their systematic screening may be rewarding in the search of better cancer therapeutic agent.

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