Secondary metabolites as DNA topoisomerase inhibitors: A new era towards designing of anticancer drugs

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ABSTRACT

A large number of secondary metabolites like alkaloids, terpenoids, polyphenols and quinones are produced by the plants. These metabolites can be utilized as natural medicines for the reason that they inhibit the activity of DNA topoisomerase which are the clinical targets for anticancer drugs. DNA topoisomerases are the cellular enzymes that change the topological state of DNA through the breaking and rejoining of DNA strands. Synthetic drugs as inhibitors of topoisomerases have been developed and used in the clinical trials but severe side effects are a serious problem for them therefore, there is a need for the development of novel plant-derived natural drugs and their analogs which may serve as appropriate inhibitors with respect to drug designing. The theme for this review is how secondary metabolites or natural products inactivate the action of DNA topoisomerases and open new avenues towards isolation and characterization of compounds for the development of novel drugs with anticancer potential.

Key words: Anticancer, DNA topoisomerases, secondary metabolities, flavonoids

INTRODUCTION

Secondary metabolites

During the course of evolution an enormous diversity has been developed in nature in the form of different plant species, insects, fungi, algae and prokaryotes. All these species coexist and interact in several ways in the environment sharing a similar biochemistry necessary for a living cell and producing a large amount of metabolites. Bearing in mind the large diversity in nature we focus on plants as a source of metabolites.[1]

Plants form an important part of our everyday diet. Their constituents and nutritional values have been intensively studied for decades. Along with the essential primary metabolites (carbohydrates, amino acids, lipids) they synthesize a wide variety of low molecular weight compounds known as the secondary metabolites. Plant secondary metabolites can be defined as the compounds that play an important role in the interaction of the plant with its environment but have no such role in maintaining the fundamental life processes in plants. Secondary metabolites often have complex and unique structures and are

stored in specific cells and or organs that are accumulated in the plant vacuoles. Production of these compounds depends on the physiological and developmental stages of the plant and is enhanced by biotic and abiotic stress.[2] Almost lacs of secondary metabolites have been discovered from the plant kingdom but only half the structures have been fully elucidated.[1] These metabolites are characterized by enormous chemical diversity and every plant species consists its own set of metabolites derived from few building blocks like C2, C9 and C5 units. Depending on the biosynthetic pathways plant secondary metabolites can be structurally divided into five major groups as polyketides, isoprenoids (terpenoids), alkaloids, phenylpropanoids and flavonoids (polyphenols). Biosynthetic pathways are often long, complex, multistep events catalyzed by various enzymes. Polyketides are produced via the acetate-mevalonate pathway, isoprenoids are derived from 5C precursor isopentenyl diphosphate (IPP) or via a classical mevalonate or non-mevalonate pathway, alkaloids are synthesized from various amino acids, phenylpropanoids having a C6-C3 unit are derived from aromatic acids and flavonoids are synthesized by the combination of phenylpropanoids and polyketides.[3]

The presence of various secondary metabolites in the plants implies multiple functions throughout the plants lifecycle. Some of the functions possessed by these metabolites are their role as mediators in the interaction of the plants with its environment (plant-insect, plant-microorganisms, plant-plant interaction), its

production as a part of plant defense system (e.g.; production of antifeedants, phytoanticipins and production of phytoalexins), its role in reproduction (insect attractant and male sterility). Secondary metabolites are also known to determine different aspect of food quality (taste, smell, color and flavor). Pigments like anthocyanins and carotenoids are important for the diversity of ornamental plants and flowers. Several secondary metabolites are used in the production of dyes, insecticides, flavors and medicines.[4]

Many of the secondary metabolites show strong biological activities like inhibition of DNA and protein synthesis, inhibition of nervous system, cardiac activity and modulation of microtubule structure.[5] Secondary metabolites thus form an extremely diverse and important class of natural products with industrial and biomedical applications and are interesting targets for drug design.[6] A number of pharmacological agents target the enzyme topoisomerase and consequently research has progressed from DNA enzymology into developmental therapeutics. The study on the basic biochemistry and molecular biology of the enzyme has lead towards its application in clinical pharmacology.

We further focus briefly on DNA topoisomerases, their types, structure and their inhibitors secondary metabolites along with their mode of action.

DNA topoisomerases: An outline of its discovery, classification, structure, and applications.

Research on DNA topoisomerase was inspired by the problem of untwining of the two parental strands of DNA by semi conservative replication. The discovery of circular DNA lead to conclude that a 'swivel' must have been introduced into a circular DNA rings to permit strand separation.[7] In 1971, Wang reported that *E. coli* extracts are capable of relaxing supercoiled DNA.[8] Initially, it was thought that relaxation of the DNA occurred by a cycle of endonucleolytic nicking and resealing of the nick by DNA ligase but subsequent purification of the enzyme however, showed that a single enzyme is capable of relaxing negatively supercoiled DNA. This enzyme was originally designated as the 'ω' protein.[9] The enzyme was later renamed as *E.coli* DNA topoisomerase I which catalyzed relaxation of negatively supercoiled DNA in the absence of any energy cofactor.[10] The discovery of *E.coli* DNA topoisomerase I led the investigators to isolate many other topoisomerases from both prokaryotes and eukaryotes. In 1972, an enzyme with activity similar to that of *E. coli* topoisomerase I was isolated from mouse embryo cells.[11] Gellert and his colleagues in 1976 identified an enzyme activity opposing *E.coli* DNA topoisomerase I by demonstrating that the enzyme (*E. coli* DNA topoisomerase II, or gyrase) catalyzed conversion of relaxed DNA into negatively supercoiled DNA in a reaction requiring ATP hydrolysis.[12]

In 1979, Liu *et al,* isolated an enzyme from bacteriophage T4-infected *E. coli* with three DNA-delay genes encoding the new enzyme (T4 DNA topoisomerase), important for T4 DNA replication. Like the *E. coli* DNA topoisomerase I or *E.coli* DNA gyrase, T4 DNA topoisomerase catalyzes relaxation of both

positive and negative supercoils in a reaction requiring ATP hydrolysis.[13-14] An archaeal type II topoisomerase activity capable of catalyzing ATP-driven relaxation and decatenation of duplex DNA circles was first discovered in *Sulfolobus shibatae* by Bergerat *et al* in 1994.[15] This protein is an A_2B_2 heterotetramer and based on the structure it is now named as DNA topoisomerase VI.[16] Further research in identification of new enzymes led to the identification of topoisomerase III from *E. coli* and yeast. [17-19] Topoisomerase IV identified in *E. coli* (ParC/ParE), shows sequence homology to gyrase and is involved in 'chromosome partitioning';[20-21] while topoisomerase V identified in the hyperthermophilic methanogen *Methanopyrus kandleri*, shows sequence homology to eukaryotic DNA topoisomerase I.[22] From the above investigation it can be assumed that DNA topoisomerases are ubiquitous enzymes found in all living organisms that is from archaebacteria to humans.[23]

DNA topoisomerases are the amazing molecular machines that manage the topological state of DNA in a cell by solving the problems associated with DNA replication, transcription and translation.[24] The enzyme accomplishes this function by transiently breaking a DNA strand and passing another strand through the transient break or by transiently breaking a pair of complementary strands and passing another double-stranded segment. These enzymes also catalyze many interconversions like catenation and decatenation, knotting and unknotting.[25]

All DNA topoisomerases share common characteristic features such as their ability to cleave and reseal the phosphodiester backbone in two successive transesterification reactions. During this transient DNA cleaving a covalent DNA-protein intermediate is formed between a tyrosine hydroxyl group of topoisomerase and DNA phosphate at break site. No energy cofactor is required for DNA breakage and rejoining activity since the bond energy is conserved in the protein-DNA backbone. Another feature is that once an enzyme- cleaved DNA complex is formed the enzyme allows the detached DNA ends to come apart, opening the gate for the passage of the DNA segments.[26]

Classification of topoisomerases

Based on the mode of cleaving DNA, topoisomerases are classified into two classes. Type I class of DNA topoisomerases change the topological state of DNA by transiently breaking one strand of DNA double helix and consequently change the linking number of DNA by one. Type II class of topoisomerases catalyze the strand passing reaction by making transient enzyme bridged double strand breaks and as a result change the linking number of DNA in the multiplies of two.[27] Type I DNA topoisomerases consist of two subfamilies type IA and type IB which are non-homologus and differ in the type of DNA adduct they form. Members of type IA subfamily forms a covalent phoshotyrosine linkage to the 5' end of DNA during catalysis and thus in earlier times were entitled as type I-5'. The members of type IB are monomeric where the protein is attached to the 3' end of DNA and thus were previously called as type I-3'. Members of type IA and type IB are included in the following Table 1.

Type II topoisomerase cleave both the strands of DNA during catalysis. The reaction is ATP dependent and these proteins cleave one DNA duplex, transport a second duplex through the break and then relegate the cleaved duplex. The type II enzymes are dimeric; the enzyme binds to duplex DNA and cleaves the opposing strands with a four base stagger. Cleavage involves covalent attachment of each subunit of the dimer to the 5' end of the DNA through the phosphotyrosine bond. Cleavage reactions cause a conformational change which pulls the cleaved duplex DNA apart to create an opening called the gate or G-segment DNA. The second region of duplex DNA from either the same molecule or a different molecule is referred as transported or T-segment thus changing the linking number by two. The members of type II are distinguished from each other with respect to the presence of different subunits. Type II enzymes from prokaryotes domains contain two different subunits and are thus heterotetrameric in structure while the members of the eukaryotic enzymes are homodimers. This groups the enzymes as type IIA and type IIB.[28-32]

The enzyme DNA topoisomerase I and II have gained wide importance in clinical research as they have been the chemotherapeutic targets. Various natural drugs have been reported to inhibit the activity of topoisomerases by acting either as topoisomerase suppressors or as poisons. Topoisomerase I suppressors correspond to compounds that inhibit the enzyme but do not stabilize the intermediate DNA- enzyme covalent complex. The interaction of the complex with the free enzyme inhibits binding of topoisomerase I to the DNA cleavage site thus preventing all subsequent steps in the catalytic cycle, while topoisomerase I poisons act after the cleavage of DNA by the enzyme and inhibit the relegation.[33] Further catalytic inhibitors of mammalian topoisomerase have been reported which target the enzyme and inhibit various processes in the cell preventing DNA damage and thus prove to be anticancer agents.[34] The role of mammalian DNA topoisomerases as molecular targets for anticancer drugs have now been recognized and investigators have carried out extensive studies on the mechanism of action of topoisomerase-targeting drugs in the cancer therapy.

Table 1: Type IA and type IB topoisomerases

Enzyme	Subfamily type	Subunit structure	Organism	Characteristics
Bacterial DNA topoisomerase I	IA	Monomer	*E. coli*	Relaxes negatively super-coiled DNA.
Bacterial DNA topoisomerase III	IA	Monomer	*E. coli*	Involved in chromosome stability and plasmid segregation.
Yeast DNA topoisomerase II	IA	Monomer	S. cerevisiae	Relaxes supercoils of both signs.
Mammalian DNA topoisomerase III α	IA	Monomer	Mammals (i. Mice) (ii. humans)	i) Required during early embryogenesis. ii) Interacts with BLM proteins.
Mammalian DNA topoisomerase III β	IA	Monomer	Humans	Interacts with RecQ family of helicases.
Eubacterial and archaeal reverse DNA gyrase	IA	Monomer	Sulfolobus acidocaldarius	Induces positive super-coiling.
Eubacterial reverse gyrase	IA	Heterodimer	Methanopyrus kandleri	Relaxes super-coiled DNA
Eukaryotic DNA topoisomerase I	IB	Monomer	Human	Bind duplex DNA and cleaves one strand forming a 3'-phoshotyrosine covalent intermediate
Poxvirus DNA topoisomerase	IB	Monomer	Vaccina	Relax either positively or negatively super-coiled DNA.
Eubacterial DNA topoisomerase V	IB	Monomer	Methanopyrus kandleri	Relax both positive and negative supercoils.
Eubacterial DNA gyrase	IIA	A2B2 heterotetramer	*E. coli*	Introduces excess negative supercoils.
Eubacterial DNA topoisomerase IV	IIA	C2E2 heterotetramer	*E. coli*	Decatenating role in replication, relaxes negative supercoils in cell.
Yeast DNA topoisomerase II	IIA	Homodimer	S. cerevisae	No super-cooling activity essential for chromosomes segregation.
Mammalian DNA topoisomerase IIα	IIA	Homodimer	Human	No super-coiling activity. Function is unknown but is expressed in only proliferating cells.
Mammalian DNA topoisomerase IIβ	IIA	Homodimer	Human	No super-coiling activity, function unknown but are expressed at equal levels in proliferating as well as quiescent cells.
Archaeal DNA topoisomerase VI	IIB	A2B2 heterotetramer	Sulfolobus shibatae	Relaxes both positive and negative supercoils

Caner is a genetic disease caused due to mutations in genes associated with cell proliferation and cell death that results in DNA damage. The understanding of cancer has revealed large numbers of exciting new targets for the development of effective therapies some of which are in clinical practice. Gene therapies propose promises for future of cancer treatment. This therapy aims in directly attacking the tumor cells but many practical obstacles need to be overcome before the therapy can fulfill its goals in clinical trials. On the other hand conventional therapy that is based on surgery, radiotherapy, chemotherapy or combination of treatments has various side effects because these drugs do not spare normal cells from their devastating actions generating toxic effects in the patients.[35] Furthermore, drug resistance in cancer cells is one of the major problems in cancer chemotherapy. In theory, drug resistance of cancer cells may arise from alterations at any step in the cell-killing pathway of the particular anticancer drug. Predominantly, resistance to various topoisomerase I and II inhibitors has been documented in tissue culture cells with respect to MDR1 over expression; reduced topoisomerase levels, drug resistant mutant topoisomerase, lengthened cell cycle time, and altered DNA repair functions.[36] Hence an altered source for the treatment of cancer is essential.

Natural drugs are thus a novel source towards the treatment of cancer as they have the ability to induce cell cycle arrest and can also repair a range of oxidative radical damages on DNA as well. There is a range of recently discovered compounds which have the ability to inhibit the action of the enzyme topoisomerases and act as promising anticancer agents. These compounds are obtained through bioactivity and mechanism directed isolation and characterization, coupled with rational drug design-based modification and analog synthesis.[37] Further are the examples of few topoisomerase targeting drugs isolated from various source.

Alkaloids as inhibitors of topoisomerase

Plant alkaloids are one of the largest diverse groups of natural products found in more than 20% of plant species. They are defined as the nitrogen containing low molecular weight compounds where the occurrence of the nitrogen ring is in an oxidative state within the heterocyclic ring. This group implies most of the bioactive metabolites which have been reported to have potent pharmacological activities.

Alkaloids are derived from the primary metabolism with amino acids as the precursors but the structural types of alkaloids have independent biosynthetic origins; like the isoquinoline alkaloids (morphine and berberine) are synthesized from tyrosine, indole alkaloids (vinblastine) are produced from tryptophan while the tropane alkaloids (cocaine, scopolamine) are derived from ornithine. These biosynthetic pathways are composed of multiple catalytic steps which form a basic structural nucleus and even alter nascent alkaloids molecules through various carbon ring modifications by multiple reactions such as hydroxylations, methylations, acetylations and glycosylations. Thus because of the above properties, alkaloids have been widely used in pharmaceutical industries in the designing of drugs.[38] Among the array of alkaloids camptothecin has been extensively used as an anticancer drug, also new alkaloids are emerging as topoisomerase inhibitors that can be used in designing of new drugs with anticancer properties.
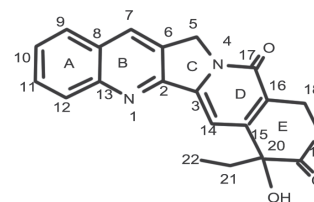
Camptothecin

Camptothecin (CPT), a potent antitumor drug was isolated for the first time from the bark and stem of the Chinese ornamental tree *Camptotheca acuminata* (Nyssaceae), also knows as the "tree of joy" [Figure 1].[39] This anticancer drug inhibits DNA topoisomerase I and hence is widely used in clinical trial as an anticancer drug. CPT has also been isolated from *Ophiorrhiza pumila* and *Mapia foetida*. It occurs in different plant parts like roots, twigs and leaves. CPT a member of quinolinoalkaloid consists of a pentacyclic ring structure which includes a pyrrole (3, 4 β) quinoline moiety and one asymmetric centre within α-hydroxyl lactone ring with 20S configuration.[40]

CPT is a potent cytotoxic drug and inhibits the DNA topoisomerase I by causing many single stranded DNA breaks while the prolonged incubation does not lead to more cleavage. CPT interferes with the breakage reunion reaction of the enzyme by trapping the reaction intermediate, the "cleavable complex" and prevents relegation.[41] This cleavable complex is stabilized and becomes non-productive in the relaxation reaction in the presence of CPT. CPT is also a highly phase specific cytotoxic

Camptotheca acuminata

Camptothecin

Figure 1: *C. acuminata* (tree of joy), structure of the anticancer drug Camptothecin
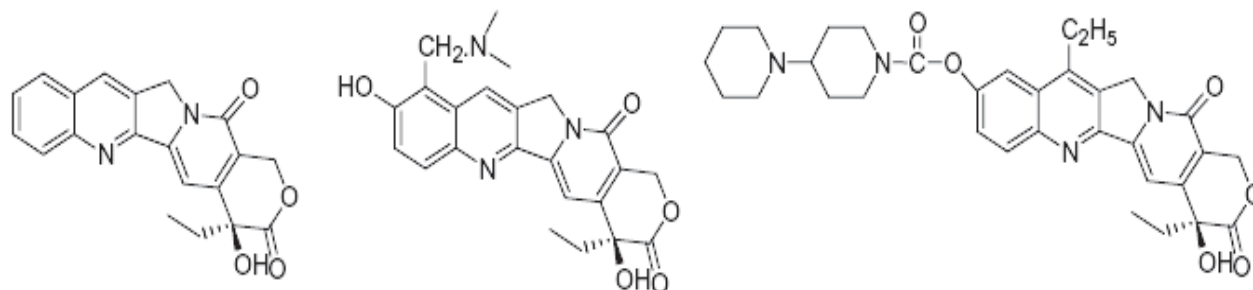
Figure 2: Camptothecin and its analogues topotecan and irinotecan.

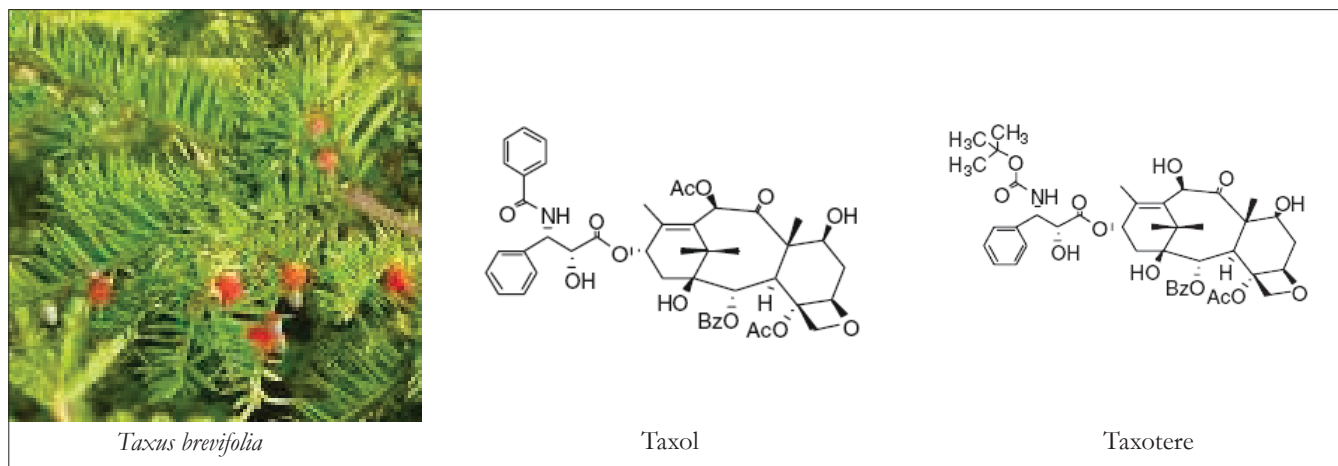*Taxus brevifolia*   Taxol   Taxotere

Figure 3: *Taxus brevifolia* and the structure of the anticancer drug taxol and taxotere

drug. It is selectively cytotoxic to S-phase cells, arrests cells in the G-2 phase and induces fragmentation of chromosomal DNA by inhibiting DNA synthesis through strand scission, thus causing cell death during the S-phase of the cycle.

Earlier reports suggested that the complete pentacyclic ring structure is essential for the antitumor activity but later on it was reported that the D ring pyrridone is required for its activity also the presence of E ring lactone form with 20S configuration gives better activity. CPT because of its severe toxicity cannot be used as a drug and hence several semi synthetic derivatives have been developed by modifying its ring structure. The most successful CPT analogues widely used in the clinical trial are topotecan and irinotecan (water-soluble) which are the obtained by modifying the A and B rings of CPT [Figure 2].

The modification at the C and D rings of CPT led to complete loss of cytotoxicity which may be because the CPT molecule loses its planarity that is supposed to be essential for enzyme-DNA-CPT ternary complex stabilization.

Campothecin and its analogues exhibit a broad spectrum of antitumor activity and represent a very promising class of agents.

CPT and its analogues shows anticancer activity against solid tumors mainly by inhibiting the action of topoisomerase I and is used in the treatment of colon and pancreatic cancer cells while its analogues are used in the treatment of breast, liver and prostrate cancer.[42-45] Alkaloids from various plants possessing topoisomerase inhibitory activity are listed in Table 2.

Terpenoids as inhibitors of topoisomerase

Terpenoids are the most structurally diverse class of plant natural products. The name terpenoid, or terpene, was derived from the compounds isolated from turpentine ("terpentin" in German). All terpenoids are derived by repetitive fusion of branched five-carbon unit based on isopentane skeleton. The isoprene units of terpenoids on thermal decomposition yield the alkene gas isoprene as a product. Appropriate chemical conditions can induce isoprene to polymerize in multiples of five carbons, generating numerous terpenoid skeletons. Thus terpenoids are often called isoprenoids.[59]

Taxol a complex polyoxygenated diterpenoid was isolated from the pacific yew, *Taxus brevifolia*, [Figure 3] by Dr. Wall and Dr.Wani.[60] Taxol as a drug has been developed by the National Cancer Institute, USA and is used in the treatment of ovarian

Table 2: List of plants and their active principles (alkaloids) acting as DNA topoisomerase inhibitors
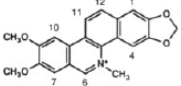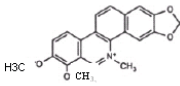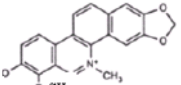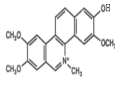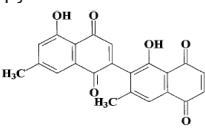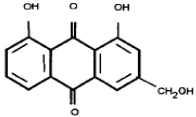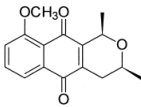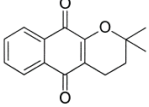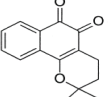
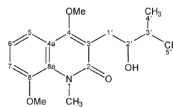| Name of plant	Type of metabolites	Name of compounds	Structure	Activity
*Zanthoxylum nitidu*m.[46] Plant part : roots	Benzophenanthridine alkaloids	Nitidine Chelerythine Isofagaridine	Nitidine Chelerythrine Isofagaridine	Compounds (i) and (iii) gave comparable inhibition of super-coiled plasmid DNA by topo I while compound (ii) partially inhibited the relaxation activity. Compound (i) stabilized the enzyme-DNA binary complex. Fagaronine inhibited the topoisomeraseI- mediated DNA relaxation, stabilized the enzyme-DNA binary complex and also induced enzyme-dependent DNA strand breaks.
		Fagaronine [47]	Fagaronine:	
Diospyros Montana.[49] Plant part : Stem bark	Bisnapthoquinoid	Diospyrin [48]	Diospyrin:	Diospyrin interacts with type I DNA topoisomerase of *Leishmania donovani* and stabilizes the cleavable complex.
Aloe vera[50] Plant part : leaves	Hydroxyanthraquinone	Aloe-emodin	Aloe-emodin	Inhibited tumor cell growth in culture cells and animal models and caused apoptotic cell death.
Eleutherine americana[52] Plant part :bulb	Pyranonapthoquinone	Eleutherin [51]	Eleutherin:	Eleutherin and β-lapachone inhibited topo II by inducing relegation and dissociation of the enzyme from DNA in the presence of ATP while β-lapachone acted as an irreversible inhibitor of topo II and α-lapachone inhibited initial non-covalent binding of topo II to DNA.
		α-lapachone	α-Lapachone:	
		β-lapachone	β-Lapachone:	

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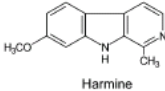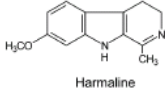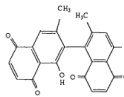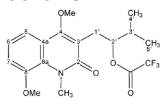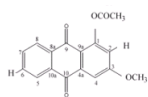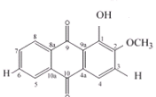
Table 2: List of plants and their active principles (alkaloids) acting as DNA topoisomerase inhibitors

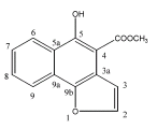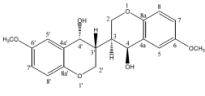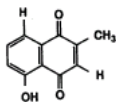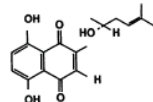| Name of plant	Type of metabolites	Name of compounds	Structure	Activity
Peganum harmala [53] Plant part : seeds	β-carbolines alkaloids	Harmine Harmaline	Harmine Harmaline	The compounds inhibited relaxation of super-coiled plasmid DNA. Harmine showed more inhibitory effect than harmaline because of its planar ring system.
Diospyros morrisana [54] Plant part: entire plant	1,2-binapthoquinone	Isodiosyprin	Isodiosyprin:	Binds directly to human topo I and not to DNA and thus inhibits the activity of the enzyme.
Lunasia amara [55] Plant part: Bark	Quinoline alkaloids	Lunacridine 2'-O-trifluoroacetyl lunacridine	Lunacridine: 2'-O-trifluoroacetyl lunacridine:	The compounds acted as the potent inhibitors of human topo II and also showed mild DNA intercalation activity.
Rubia cordifolia [56] Plant part: roots	Anthraquinones, Benzopyran	Furomollugin 1-acetoxy-3methoxy-9,10-anthraquinone Alizarin 2-methyl-ether) benzopyran derivative	Furomollugin: 1-acetoxy-3-methoxy-9,10-anthraquinone: Alizarin 2-methyl-ether): Benzopyran derivative	Compounds i, ii and iii showed strong inhibitory activity of DNA topoisomerase I at higher concentrations. While the benzopyran derivatives and furomollugin inhibited the activity of DNA topo II.

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Table 2: List of plants and their active principles (alkaloids) acting as DNA topoisomerase inhibitors

Name of plant	Type of metabolites	Name of compounds	Structure	Activity
Dionaea muscipula.[57]	Naphthoquinones	Plumbagin	Plumbagin	The compounds act as topo II poisons by inducing mammalian DNA topo II mediated DNA cleavage through the formation of reversible, cleavable complex.
		Shikonin	Shikonin	
Tabernaemontana divericata.[58]	Alkaloids	Ethyl acetate extract		The ethyl acetate extracts selectively inhibited the topo II activity in the relaxation assay.

cancer, metastatic breast and lung cancer and Kaposi's sarcoma. It has a basic pentadecane, tetracyclic ring system and a N-benzoyl-b-phenylisoserine side chain attached at the C-13 hydroxyl as an ester linkage. This side chain is essential for the anticancer activity. [61] Taxol has several side effects like numbness, nausea, tingling in toes and also reduction in the W.B.C's as it affects the bone marrow and hence cannot be used directly. Taxotere one of the semisynthetic derivative of taxol is a potent anticancer drug as it has improved water solubility and acts at the microtubules enhancing polymerization of tubulin into stable microtubule bundles leading to apoptosis. It is used for the treatment of breast cancer and non small cell lung cancer.[62,63] Terpenoids from various plants possessing topoisomerase inhibitory activity are listed in Table 3

Flavonoids as inhibitors of topoisomerases

Flavonoids are low molecular weight polyphenolic compounds, ubiquitous in the plant kingdom. These polyphenolic compounds have variable chemical structures and are found in vegetables, fruits, grains, tree barks, roots, stems, flowers and also in wine. This diverse group of secondary metabolites has a vast array of biological functions. Flavonoids are classified into several subclasses such as isoflavonoids, chalcones, flavanones, flavones, dihydroflavonols, flavonols, anthocyanidins and catechins.[69-72] The basic structural feature of flavonoids compound consist of the flavone nucleus composed of two benzene rings A and B linked through a heterocyclic pyrane C ring. Based on the basis of the position of the benzenoid ring B the flavonoids class is divided into flavonoids (2-position) and isoflavonoids (three -position). [73] Flavonoids have wide range of biological activities like antiviral, antiinflammatory, antitumor, antimicrobial, estrogenic, antiestrogenic and antioxidant, mutagenic and antimutagenic because of which they are emerging as nutraceuticals in pharmaceutical industries.[74]
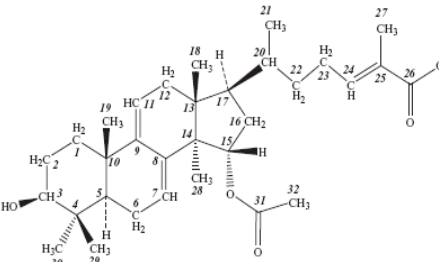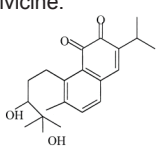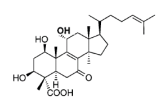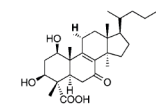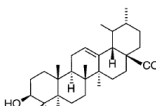
Austin *et al,* studied the inhibitory activities of plant derived flavonoids baicalein, quercetin, quercetagetin and myricetin and two catechins (-) epicatechin gallate and (-) epigallocatechin gallate isolated from *Camellia sinensis* on mammalian DNA topoisomerase II. They reported that the flavonoids quercetin, quercetagetin, myricetin and baicalein altered the linking number of DNA as compared to the control while catechin derivatives did not show any effect on the DNA topology. The decatenation assay of topoisomerase II showed that quercetin inhibited the activity of topoisomerase II while catechins showed less inhibitory activity.[75]

Constantinour and co-workers studied the inhibitory activity of topoisomerase I and II by performing topoisomerase I relaxation assay, topoisomerase II unknotting assay and plasmid linearization assay. The activity of 20 flavonoid compounds was assessed and it was reported that myricetin, quercetin, fisetin, and morin inhibited both enzymes, while phloretin, kaempferol, and 4', 6, 7-trihydroxyisoflavone inhibited topo II without inhibiting topo I. Topoisomerase II inhibiting flavonoids can function as topo II poisons, antagonists, or both depending on the position of hydroxyl groups in the A and B rings of the molecule. So, flavonoid antagonists may bind with a spatial orientation that neither interferes with the DNA cleavage/relegation equilibrium, nor opposes the DNA strand-passage step of the reaction but rather they inhibit enzymatic turnover through a mechanism requiring ATP hydrolysis. Flavonoid poisons, on the other hand, because of a different spatial arrangement, may stabilize the (normally transient) DNA-enzyme complex and favor the DNA cleavage component of the reaction.[76]

Boege *et al,* reported that quercetin and related natural flavone derivatives, such as acacetin, apigenin, kaempferol, and morin, stabilize the covalent DNA topoisomerase I-DNA post-cleavage complex by inhibiting the relegation process.[77]

Table 3: List of plants and their active principles (terpenoids) acting as DNA topoisomerase inhibitors

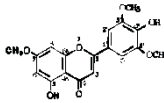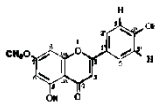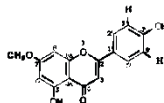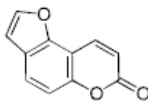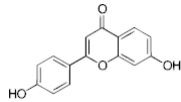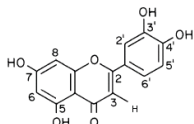| Name of plant	Type of metabolites	Name of compounds	Structure	Activity
Euphorbia kansui [64] Plant part: Roots	Terpenes	Ingenol 5-acetate ingenol 20-O-(2'E', 4'Z) - ingenol		The compounds acted as potent inhibitors of topoisomerase II.
Ganoderma amboinense[65]	Lanostanoid triterpenes	Ganoderic acid X	Ganoderic acid X	Ganoderic acid X inhibited the activity of both the enzymes topo I and topo IIα in a dose-dependent manner.
Salvia prionitis[66]	Diterpenoid quinone	Salvicine	Salvicine:	The compound acts on the enzyme by trapping the DNA topo II complex. It shows strong inhibitory activity against the enzyme in both the relaxation and decatenation assay.
Fomitella fraxinea Tabebuia caraiba Campsis radicans[68]	triterpenoids	Fomitellic acid A (FA-A)[67]	FA-A	The compounds showed significant effect on the catalytic activity of human DNA topoisomerase I and II. FA-A inhibited the activity of topo I and II in a dose dependent manner while ursolic acid acted at a higher concentration.
		Fomitellic acid B (FA-B)	FA-B	
		Ursolic acid	Ursolic acid	

Bernard *et al*, reported that the glycosylated flavones present in the cotton seed flour were selective poisons of E.coli topoisomerase IV. Among the flavones rutin was the most potent in stimulating topoisomerase IV- dependent DNA cleavage and it also blocked the catalytic activity of topoisomerase IV.[78] Flavonoids from various plants possessing topoisomerase inhibitory activity are listed in Table 4.

Cantero *et al*, conducted a comparative study of luteolin and quercetin to check its effect on topoisomerase II activity from Chinese hamster ovary AA8 cells and found that both the compounds inhibited topoisomerase II catalytic activity resulting in extraordinarily high yields of metaphases showing diplochromosomes. The studies on luteolin and quercetin showed that the compound acts as a lead for cancer treatment.[83]

Lopez-Lazaro reported that the dietary flavonoids genistein and luteolin as topoisomerase I and II poisons in the cell based assay. They also reported that the flavonoids functioned as the catalytic inhibitors in K562 leukemia cells.[84]

Vega *et al*, reported the activity of the flavonoids and flavonoid fraction from *Annona dioica*. The methanolic extracts of the leaves of *Annona dioca* showed the presence of four flavonoids kaempferol (1), 3-O-[3", 6"-di-O-p-hydroxycinnamoyl]-β-galactopyranosyl-kaempferol(2),6"-O-phydroxycinnamoyl-β-galactopyranosyl-kaempferol(3) and 3-O-β-galactopyranosyl-kaempferol (4). They observed that all the tested flavonoids except the flavonoid fraction showed inhibitory relaxation effects as compared to quercetin which was used as the positive control. The inhibitory effects on DNA-topoisomerase II-α was also evaluated, by the relaxation assays using supercoiled pBR322

Table 4: List of plants and their active principles (flavonoids) acting as DNA topoisomerase inhibitors

Name of plant	Type of metabolites	Name of compounds	Structure	Activity
Lethedon tannaensis[79]	Flavones	7,3′5′-tri-O-methyltricetin	7,3′5′-tri-O-methyltricetin	The compounds significantly inhibited the activity of topoisomerase I.
		velutin	Velutin	
		genkwanin	Genkwanin	
Psoralea corylifolia[80] Plant part: seeds	Furanocoumarin Isoflavonoid	Bakuchicin	Bakuchicin	The compounds inhibited the activity of DNA topoisomerase II
		Daidzein	Daidzein	
Vitex negundo[82]	Flavonoids	Luteolin[81]	Luteolin	Acts as a potent inhibitor of eukaryotic DNA topoisomerase I. It intercalates with DNA and does not bind to the minor groove and also stabilizes the cleavable complex.

plasmid DNA in the presence of ATP. They observed that all assayed flavonoids, (except 4) including the FF fraction showed significant inhibitory effect as compared to etoposide.[85]

Stilbenes as inhibitors of topoisomerase

Stilbenes are the naturally occurring, low molecular weight compounds found in a wide range of plant sources. These compounds are synthesized via the phenylpropanoid pathway. Upon attack by pathogens, the plant host activates the phenylpropanoid pathway producing stilbenes. Stilbenes act as natural protective mediators to defend the plants against the viral and microbial attack, exposure to ultraviolet radiation and diseases. These compounds have some structural similarities with estrogen. Stilbenes exists in the stereo isomeric forms like the E and Z forms, depending on the position of where the functional groups are attached in relation to one another on either side of the double bond. Combretastatins, piceatannol, pinosylvin, rhapontigenin, pterostilbene and resveratrol are some of the naturally occurring stilbenes. Of these combretastatins

and resveratrol has been extensively studied as it has been used in anticancer, antioxidant and anti-inflammatory activities.[86]

In 1982 Pettit *et al*, isolated combretastatins from the bark of the South African tree *Combretum caffrum*. Combretastatin are the first members of a series of biologically active bibenzyls, stilbenes and phenantherenes.[87-89] Combretastatins A-1 and A-4 are stilbene derivatives having two phenyl rings separated by a C-C double bond [Figure 4]. Ring-A has three methoxy groups in 3,4,5-positions while in ring B one hydroxyl group is at the C-3 position and one methoxy group at the C-4 position. Combretastatin A-4 [20, cis-1-(3, 4, 5-trimethoxyphenyl)-2-(30-hydroxy-40-methoxy phenyl) ethene], a simple stilbene has been found to be a potent cytotoxic agent that strongly inhibits the polymerization of brain tubulin by binding to the colchicine site.[90] The compound is active against colon, lung and leukemia cancers.

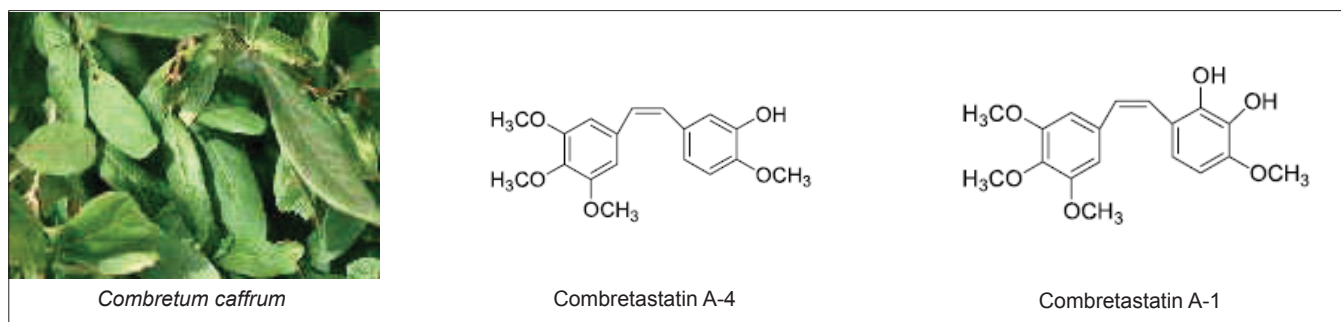*In vitro* studies have shown that CA-4 competes with colchicine

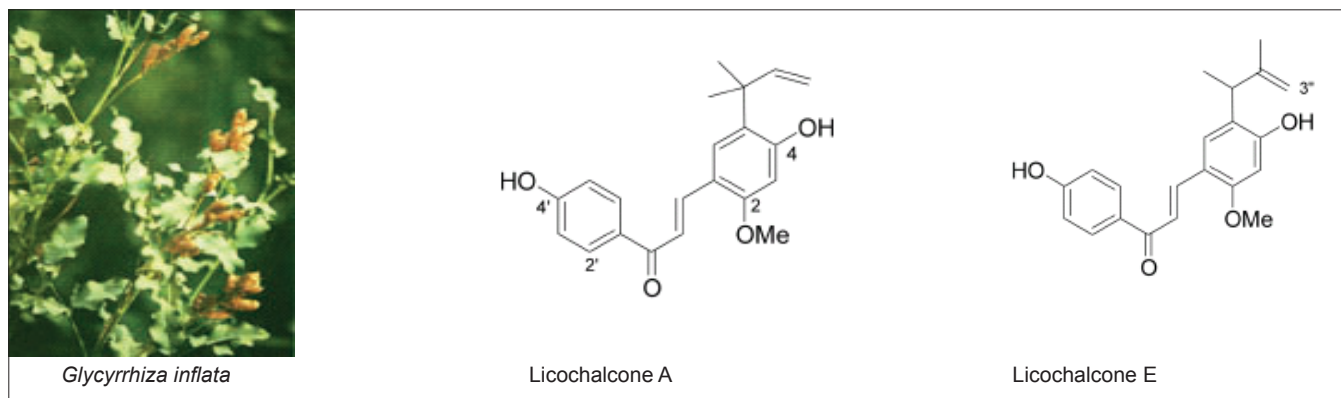**Figure 4:** *Combretum caffrum* and its stilbene Combretastatin A-4

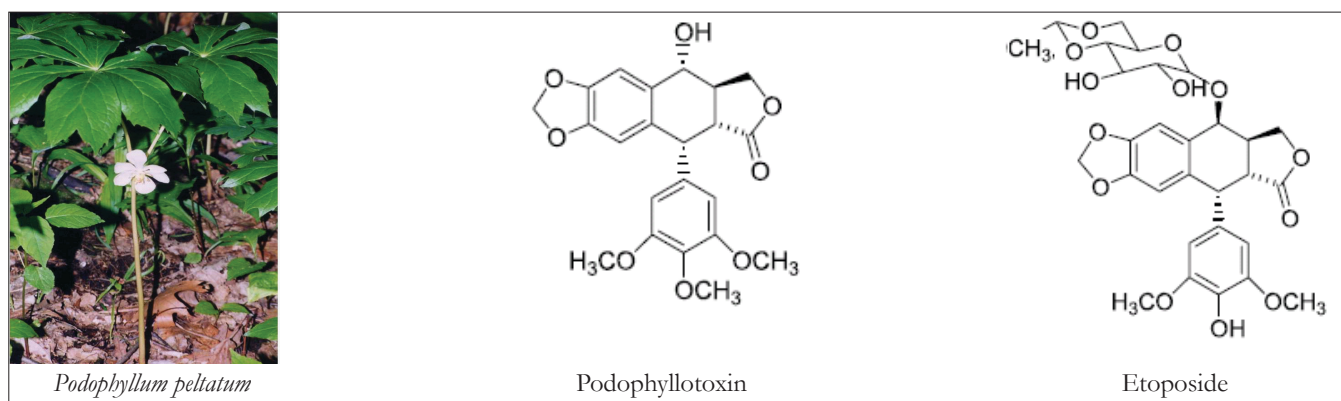**Figure 5:** *Glycyrrhiza inflata* and the structure of the licochalcones A and E

Figure 6: *Podophyllum peltatum* and the lignan podophyllotoxin and its analogue etoposide

for binding sites on tubulin. Hence, it is a member of the colchicine-like inhibitors of microtubulin.[91] Further, by making modifications in the structure of combretastatin and by developing its derivatives new drugs with activities similar to or better than combretastatin can be obtained. Thus combretastatin or its analogues may come up as anticancer drugs of choice in near future. Stilbenes from various plants possessing topoisomerase inhibitory activity are listed in Table 5.

Chalcones as inhibitors of DNA topoisomerase

Chalcones are substances widely present in the plant and are the intermediates in the biosynthesis of flavonoids. They have a wide range of biological activities like antibacterial, antifungal, antitumor and antiinflammatory. Structurally they are aromatic ketones which act as a central core for a variety of biological activities. Further are few chalcones and its derivatives with anticancer properties.

Yoon *et al,* isolated retrochalcones (compounds that are structurally distinguished from normal chalcones by their lack of oxygen molecule at the C-2' and C-6' positions) from the roots of *Glycyrrhiza inflata* (Leguminosae) [Figure 5]. The activity of DNA topoisomerase I was determined by measuring the relaxation of supercoiled DNA pBR322. The licochalcones A and E inhibited the activity of topoisomerase I in a dose dependent manner. The compounds showed potent inhibition of topoisomerase

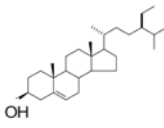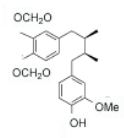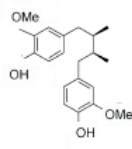## Table 5: List of plants and their active principles (stilbenes) acting as DNA topoisomerase inhibitors
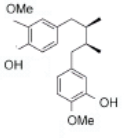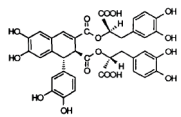
Name of plant	Type of metabolites	Name of compounds	Structure	Activity
Polygonum multiflorum[92]	Stilbenes Sterols	Physcion β-sitosterol and their glucopyranoside and glucoside.	β- sitosterol	The compound β- sitosterol showed strong inhibitory activity against topoisomerase I and II.
Kobresia nepalensinol[93]	Stilbenoids	Nepalensinols D, E, F and G		Nepalensinol F showed inhibitory action against the decatenation activity of human topoisomerase II on kinetoplast DNA than the remaining compounds.

Table 6: List of plants and their active principles (lignans) acting as DNA topoisomerase inhibitors

Name of plant	Type of metabolites	Name of compounds	Structure	Activity
Machilus thunbergii [103] Plant part: Bark	Lignans	Erythri-austrobailignan Meso-dihydroguaiaretic acid Nectandrin B	Erythri-austrobailignan Meso-dihydroguaiaretic acid Nectandrin B	All the compounds showed strong inhibitory activity of topoisomerase I while meso-dihydroguaiaretic acid also showed inhibitory activity of topoisomerase II.
Arnebia euchroma[104]	Lignan	Rabdosiin	Rabdosiin	The compound showed potent inhibitory activity of against topoisomerase II

I and thus can serve as lead structures for the development of anticancer drugs.[94]

Lignans as topoisomerase inhibitors

Another important addition to the anticancer drug armamentarium is the class of lignans. Lignans are a family of natural products originated as secondary metabolites through the shikimic acid pathway. They are formed by the combination of two phenylpropane units and constitute a complex family of skeletons and characteristic chemical functions, which can be subdivided

into four groups: Lignans, neolignans, oxyneolignans and trimers, higher analogues and mixed lignanoids Among the lignans, cyclolignans present a carbocycle between both phenylpropane units, created by two single carbon-carbon bonds through the side chains, one of them between the β-β' positions. The aryltetralin structure of podophyllotoxin belongs to cyclolignans.[95]

Podophyllotoxin (PDT), a bioactive lignan, was first isolated by Podwyssotzki in 1880 from the North American plant *Podophyllum peltatum* [Figure 6]. This compound chemically an

aryltetralin lignan has a lactone ring.[96] These PDT lignans block the catalytic activity of DNA topoisomerase II by stabilizing a cleavage enzyme-DNA complex in which the DNA is cleaved and covalently linked to the enzyme.[97-98] Podophyllotoxin shows strong cytotoxic activity against various cancer cell lines. It is effective in the treatment of Wilms tumors, various genital tumors and in non- Hodgkin's and other lymphomas and lung cancer.[99-101] The drug when used in the treatment of human neoplasia showed severe side effects and hence could not be used as such in further treatment. Therefore various modifications were performed to obtain a more potent and less toxic drug. This lead to the synthesis of two new analoges etoposide and teniposide which are widely used for the treatment of various cancers.[102] Lignans from various plants possessing topoisomerase inhibitory activity are listed in Table 6.

CONCLUSIONS

Secondary metabolites isolated from the plants have been a principal source of most effectual conventional drugs for the treatment of various forms of diseases especially different forms of cancers. Many times the legitimate compounds isolated from the plants may not serve as the novel drug because of the side effects (e.g.: Taxol, CPT) and hence it leads to the development of potential novel analogue with better activity.

The enzyme, essential for the topological changes in DNA that is DNA topoisomerase also called the double-edged sword are clinical targets in cancer therapy. This review summarizes the clinical implications of various inhibitors of DNA topoisomerase because of their strong antitumour activities. Natural drugs and their analogues have been extensively characterized with respect to their mode of action, application to the functional analysis of the enzyme to various genetic processes in the cell and especially their allegation in cancer chemotherapy. Supplementary research in the isolation, characterization of plant based natural drug and their mode of action may lead to the development and designing of various drugs with anticancer potential. Thus research in metaboliomics may open new avenues in development on drugs targeting the enzyme and preventing the genetic illness.

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