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ALKALOID DRUGS PRODUCTION BY METABOLIC ENGINEERING OF MICROBES AND THEIR MEDICAL APPLICATIONS: A REWIEW

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ABSTRACT

Alkaloids based pharmaceutical products are mainly produced by some plants species and further proceeds to fermentation in bioreactors while due to some limitations; it's not a well defined way for alkaloids production. The most important alkaloids are benzylisoquinoline alkaloids, ergot alkaloids, quinoline alkaloids, monoterpene indole alkaloids, tetrahydroisoquinoline alkaloids, phenylethylisoquinoline alkaloids, phenylethylamines alkaloids and pyrroloquinoline alkaloids. While endophytic fungi in plants are also good sources of alkaloids drugs, which showed excellent biological and medical properties including anticancer, insecticidal, cytotoxic and antimicrobial activities. Now days, plant pathways, in order to generate alkaloid-derived pharmaceutical products, are introduced into microbes. Many genes coding for specific enzymes, involved in biosynthesis of alkaloids, are sequenced and introduce into microbes by recombinant DNA technology. In this review, we examine main sources of alkaloids based drugs and advance methods for their better production. Furthermore, we investigate challenges, which cause hindrance in microbial production of alkaloids-derived drugs that must be defeated.

KEYWORDS: Alkaloids, Endophytic fungi, Biosynthesis, Microbial production, Biological properties.

INTRODUCTION

Plants are the major source of secondary metabolites including several types of drugs; these metabolites are not required for energy and primary metabolism. These metabolites have importance for the plants that produce them as they provide them ecological fitness.[1] *In vitro* systems including bioreactors are possibly used to produce these valuable drugs (Fig.1). Organ cultures, callus culture, suspension cell culture and large scale fermentation of suspended cells were productively well-known over the last 40 years.[2] Microbes are also used for the production of these plant drugs now days.[3] By using advanced metabolic engineering techniques, plant metabolic pathways are introduced into microbes (0.5–3 h doubling time) for the production of drugs. Compounds, for which biosynthetic enzymes are known, are produced by introducing genes encoding for that specific enzyme into microbes such as *Escherichia coli* and *Saccharomyces cerevisia*.[4] In 1989, plant gene encoding for phenylalanine ammonium lyase (PAL) was introduced in *Escherichia coli* for the production of important intermediates of flavonoids and some phenylpropanoids biosynthesis pathways. Artemisinic acid, that is required for the production of antimalarial artemisinin, was produced by microbial titer of 25g/L in 2014.[5] Because of the multifaceted polycyclic nature of alkaloids compounds, it is difficult to modify structures of these compounds. Although chemical methods for the modification of alkaloids are well known but these are successfully modified by biotransformation. Plants and microbial biotransformation of alkaloids was freshly reviewed in which they make availability summary of the advancement of alkaloid biotransformations from mid-1980s to 2002.[6]

Endophytic fungi, defined as fungi whose lifecycle rely upon host plants in which they colonize intra or inter cellularly, are also widely used for alkaloids production. Almost every plant species exhibited these fungi as these are the important part of plant micro-ecosystem. Over one million fungal endophytes are found in nature. Palcitaxel (taxol) was discovered by *Taxomyces andreanae*, an endophytic fungus, from *Taxus brevifolia* in 1993**.** Many compounds including podophyllotoxin, hypericin and camptothecin were discovered consecutively from endophytic fungi.[7] Many secondary metabolites can also be produced by endophytic fungi. From these secondary metabolites, alkaloids are the important one. Alkaloids have both chemical as well as biological properties such as anticancer, antiviral and antifungal activities. Scientists emphasized on using endophytic fungi as they are important source of leading drugs for example Camptotheci as shown in table 1.[8,9] This review describes the production of alkaloids drugs by means of microbes, especially alkaloid-producing endophytic fungi according to literature.

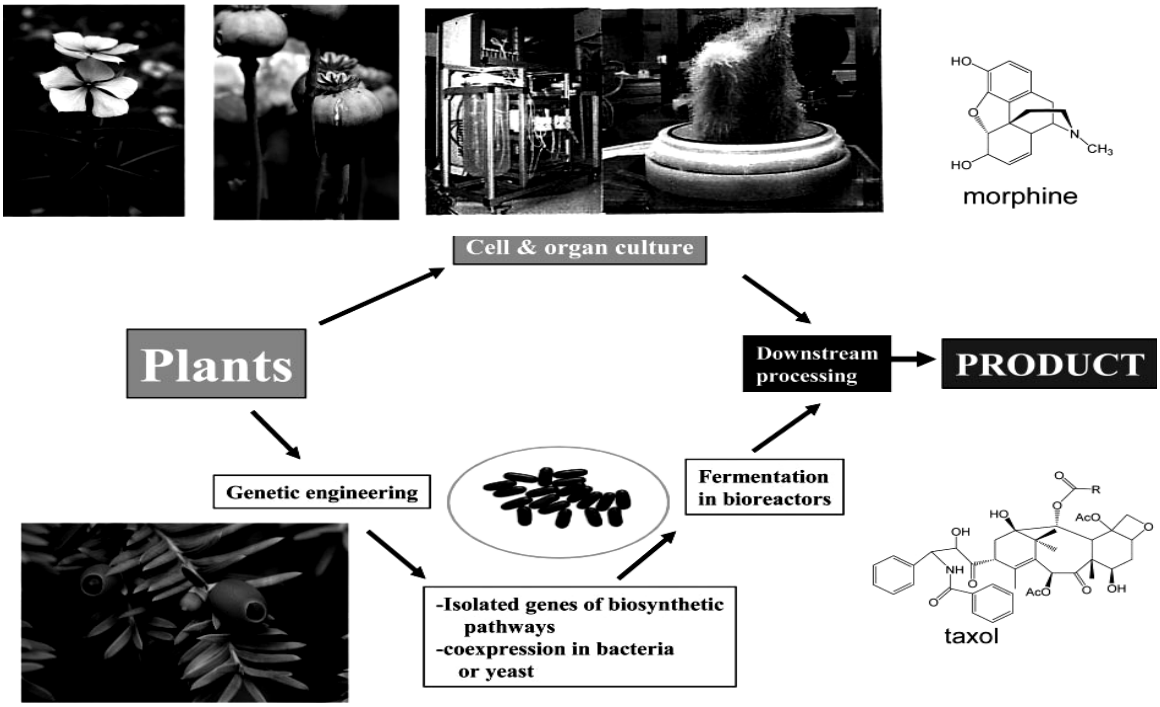

Fig. 1: Some strategies for the production of secondary metabolites

Table 1: Camptothecin-producting endophytic fungi species

Grass	Endophytic species	References
Nethapodytes fortida	*Entrophospora infrequens*	[10]
Apodytes dimidiata	*Fusarium solani*	[11]
Nethapodytes fortida	*Fusarium solani*	[12]
Nethapodytes fortida	*Neurospora sp.*	[13]
Nethapodytes fortida	*Nodulisiporium sp.*	[14]

Bioproduction of alkaloids

To catalyze the biosynthesis of secondary metabolites, substrate-specific enzymes are required, while enzymes (such as glycosidases, esterases) for breakdown process are not substrate specific. The nitrogenous part of alkaloid drives from amino acids (such as tryptamine, tyrosine, ornithine, lysine and phenylethylamine). Firstly, decarboxylation of amino acids occurred by decarboxylases enzymes, along with tryptamine, phenylethylamine, putrescine, cadaverine and dopamine. Further reaction takes place between amino group of amines and aldehyde molecules, under physiological conditions both of these molecules form a Schiff's base. Other reactions that take place include the addition of functional groups (methylene dioxy, methyl, hydroxyl-groups) oxidation or reduction of double bonds, ring closure and modifications of OH-groups such as methylation, esterification, glycosylation.[15] Finding of enzymes involved in the biosynthesis of alkaloids is a continuing practice. This search has been booming for a number of alkaloids groups such as furanoquinoline alkaloids, tropane-, ergot-, monoterpene indole-, protoberberine-, Nicotiana-, pyrrolizidine, purine-, Taxus- and morphinane.[16] However, much effort still desires to be done in order to determine biosynthesis of many alkaloids. Pathways that involve in the SM biosynthesis have been explored in a few plants and it is assumed that all plants have same pathways in order to produce SM. But this supposition still wants to be tested.

Genes for alkaloid biosynthesis

After separation and purification of enzymes, involved in alkaloids biosynthesis, genes coding for them could be sequenced and by using primers cDNA could be synthesized. cDNA clones, coding for enzymes that involved in alkaloid biosynthesis, could be produced. Then corresponding genes could be expressed in recombinant system. Strictosidine synthase (STR), involved in the biosynthesis of Monoterpene indole, was the first alkaloid gene to be cloned.[17]

Phenylethylamines alkaloids

Phenylethylamines, such as dopamine responsible for production of complex alkaloids, are synthesized by decarboxylation of phenylalanine in engineered microbes.[18] Ephedrine and pseudoephedrine, the amphetamine-derived drugs, are produced by using yeast. Phenylacetylcarbinol is produced by feeding yeast with benzaldehyde, which is ultimately transformed to final product. Metabolic engineering and enzymes are involved in the bioproduction of phenylethylamine-derived pharmaceuticals.[19]

Phenylethylisoquinoline alkaloids

The gallows of phenylethylisoquinoline alkaloids are produced by Condensation of 4-hydroxydihydrocinnamaldehyde and dopamine .The biosynthetic pathway for colchicine from 4-hydroxydihydrocinnamaldehyde and dopamine is not known. Oxidation of autumnaline, P450-dependent, is the only illuminate step for their synthesis.[20] Biosynthetic enzymes for colchicines production, involved in different catalytic reaction including methylation and demethylation, ring formation and cleavage, amide hydrolysis and formation and oxidation, are discovered by genome sequencing of *G. superb.*

Quinoline alkaloids

Quinoline alkaloids are formed in results of rearrangement of the indole ring in (*S*)-strictosidine to a quinoline ring. Commercial source of quinoline alkaloids is *Cinchona* bark. From quinoline alkaloids, Camptothecin is one of the most powerful cytotoxic compounds.[19] Usually researchers extract it from the roots of *Nothapodytes fortida* is one of the good source of quinoline, in which CPT highest concentration is 0.3-0.5%.[21] Now days, CPT is obtained from microbes, especially from endophytic fungi. Microbes are not engineered to produce quinoline; reason behind this is limited knowledge of their biosynthetic pathways. cinchoninone: NADPH oxidoreductase is only one enzyme which is used in quinine semisynthesis.

Benzylisoquinoline alkaloids

Benzylisoquinoline alkaloids are the major groups of several drugs that have medical importance, such as thebaine, papaverine, morphine, sanguinarine, codeine or berberine. Protoberberine, aporphine, morphinan, tetrahydroisoquinoline and benzophenanthridine alkaloids are of structural types and have been synthesized (Fig. 2). X-ray data is also available for few enzymes such as norcoclaurine synthase[22] and berberine bridge enzyme (BBE). (*S*)-norcoclaurine, form in the result of condensation process of 4-HPAA and dopamine, converted to (*S*)-reticuline which is the common originator of all benzylisoquinoline alkaloids. Microbes are engineered for the production of BIAs and it has been extremely flourishing in the last eight years.[4] Simple sugars are involved in microbial production of simple BIAs, including reticuline, norlaudanosoline and norcoclaurine, while more complex BIAs, including noscapine, magnoflorine, canadine and morphine and have been produced from simple sugars or advanced intermediates. Bisbenzylisoquinoline alkaloids (BBIAs) production results in the dimerization of two simple BIAs with the help of one or two ether linkages. These synthetic BBIA cores are associated by means of a polymethylene chain.

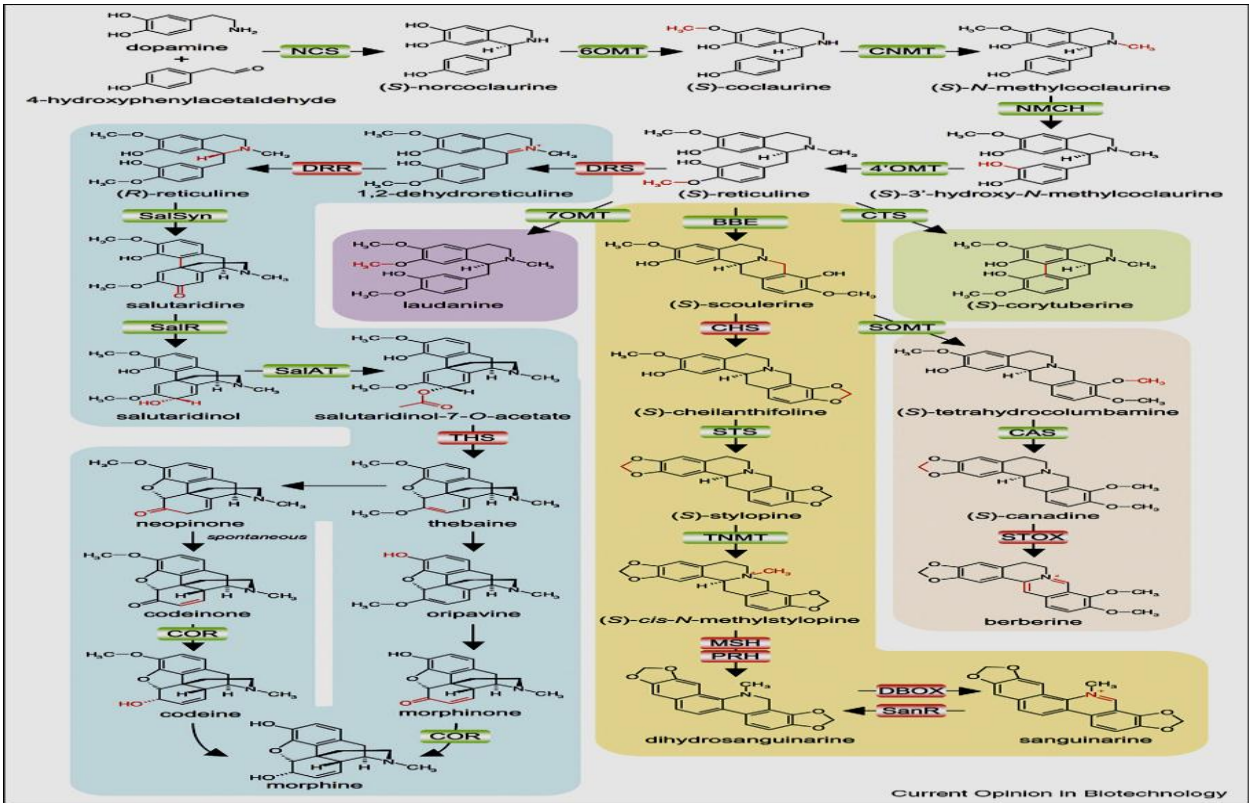

Fig. 2: Biosynthesis of benzylisoquinoline alkaloids (BIAs)

Cisatracurium, Mivacurium and atracurium, synthetic BBIA-based drugs, have been accepted as muscle relaxants. Atracurium is an isomeric combination, while cisatracurium has fewer side effects and greater pharmacodynamics in comparison to atracurium. (*R*)-tetrahydropapaverine is obtained through optical

resolution of (*R*,*S*)-tetrahydropapaverine and synthesized cisatracurium in two steps while ~60% of the starting material discarded. Although (*S*)-tetrahydropapaverine is the natural product, a single enzyme could be engineered to produce non-natural (*R*)-tetrahydropapaverine from (*R*)-norlaudanosoline. Spontaneous Pictet–Spengler condensation of 3,4-dihydroxyphenylacetaldehyde and dopamine, starting from supplied glucose, leads to the production of Racemic (*R*,*S*)-norlaudanosoline in *E.* coli.[19] Tetrahydropapaverine are produced by expressing *Glaucium flavum* GFLOMT2, that expressed in results of feeding (*R*,*S*)-norlaudanosoline to *E. coli*.[23] It is hypothesized that both *R* and *S* isomers are converted by enzyme as no norlaudanosoline was found at the end of reaction. The pathway leading to protoberberine, morphinane and tetrahydroisoquinoline alkaloids has been explained and most of the genes responsible for their synthesis have been cloned and characterized (Table 2).

Table 2: Cloned genes and characterized enzymes involved in the biosynthesis of benzylisoquinoline alkaloids

Enzymes	References
Norcoclaurine synthase (NCS)	[24]
Coclaurine *N*-methyltransferase (CNMT)	[25]
N-methylcoclaurine 3'-hydroxylase (Cyp80B)	[26]
Reticuline 7-*O*-methyltransferase (7OMT)	[27]
Cheilanthifoline synthase (Cyp719A5)	[28]
Tetrahydroprotoberberine *N*-methyltransferase (TNMT)	[29]
Scoulerine 9-*O*-methyltransferase (SOMT)	[30]
Salutaridine reductase (SalR)	[31]
Codeinone reductase (COR)	[32]
Cyp80G2	[28]
Salutaridinol 7-*O*-acetyltransferase (SalAT)	[33]
Columbamine *O*-methyltransferase (CoOMT)	[34]
Cyp719A1	[26]
Stylopine synthase (Cyp719A2, 3)	[35]
Berberine bridge enzyme (BBE)	[36]
3-Hydroxy-*N*-methylcoclaurine4-*O*-Methyltransferase (4'OMT)	[37]
Berbamunine synthase (Cyp80A1)	[38]
Norcoclaurine 6-*O*-methyltransferase (6OMT)	[27]

Ergot alkaloids

Indole alkaloids, ergot alkaloid type, are produced by *Claviceps purpureus* and other fungi. These alkaloids have well-defined actions in neuronal signal transduction (such as found in ergometrine, LSD, ergotamine). Ergot alkaloids have also been recognized in a few plants belongs to family Convolvulaceae. An endophytic fungus, lives in symbiosis relation with its host plant, is responsible for the production of ergot alkaloids in *Ipomoe*.[39] Synthetic peptides and lysergic acid are responsible for ergot alkaloids production, mainly four ergopeptides (bromocriptine, ergotamine, ergoloid mesylate and dihydroergotamine). Paspalic acid and lysergic acid hydroxyethylamide (Fig. 3), used to semi synthesize lysergic acid, are gained from fermentation broth of Claviceps.[40] Lysergic acid is produced in a non ergot producing microbe to avoid contamination, caused by other similar metabolites, in order to produce ergot-based pharmaceuticals. Eight enzymes are involved in semi synthesis of lysergic acid by converting dimethylallyl pyrophosphate and tryptophan into lysergic acid**.**

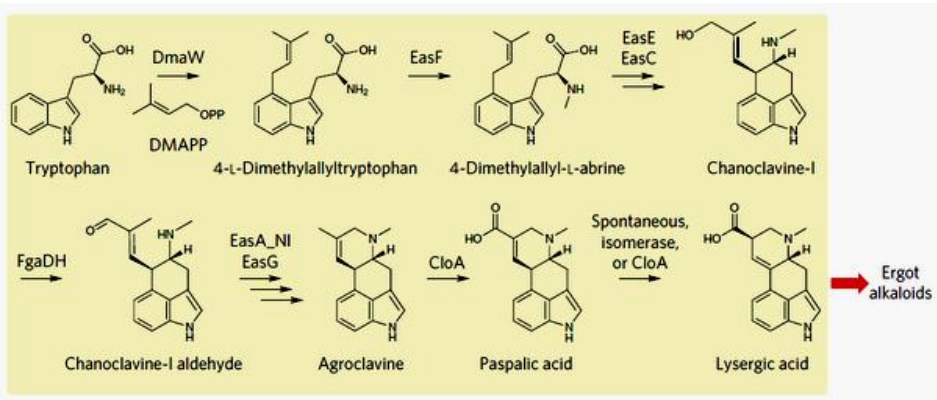

Fig. 3: Semisynthesis of lyserfic acid for the production of ergot alkaloids

Tetrahydroisoquinoline alkaloids

Tetrahydroisoquinoline alkaloids are produced by Pictet–Spengler reaction in which phenylethylamine is condensed with an aldehyde. *Pseudomonas fluorescens* produced cyanosafracin B, which is responsible for trabectedin semisynthesis.[41] (1*S*)-phenyl-tetrahydroisoquinoline is an essential alkaloid intermediate for the production of solifenacin. Industrially, it is produced in three chemical steps,during which >50% of the material is discarded. Biologically, Pictet–Spenglerase norcoclaurine synthase (NCS) is used to enzymatically combine the benzaldehyde and phenylethylamine to produce (1*S*)-phenyl-tetrahydroisoquinoline. Dopamine and 4-hydroxyphe-nylacetaldehyde (4-HPAA) are condensed by wild-type NCS to form (*S*)-norcoclaurine. However, neither phenylethylamine is condensed by 4-HPAA nor dopamine by benzaldehyde.[42] Commercial synthesis of solifenacin is accelerated by engineered NCS, in order to generate (1*S*)-phenyl-tetrahydroisoquinoline.

Tryptamines and β-carbolines

Tryptamines are formed in the result of decarboxylation of tryptophan. However, this decarboxylation process also leads to the production of eight simple tryptamine derivatives, from which six 'triptans' such as rizatriptan and sumatriptan are on the market. Some derivates of tryptamine, such as serotonin and tryptamine, are involved in microbial productions[43] while some of these derivatives have also been formed as end products. However, these simple tryptamine derivatives can also be competently generated by chemical means. β-carbolines are synthesized under condensation process between tryptamine and aldehydes (Fig. 4). Tadalafil is the only permitted β-carboline drug on the market that used as a vasodilator for male erectile dysfunction. In β-carboline scaffold *cis* arrangements of the piperazinedione moieties and benzodioxole are the structural features of tadalafil. Commercially, tadalafil is synthesized in three steps that starting from commercially available materials and leverage. While desired *trans* isomer stays in solution *and cis* isomer precipitated out of the reaction.[44]

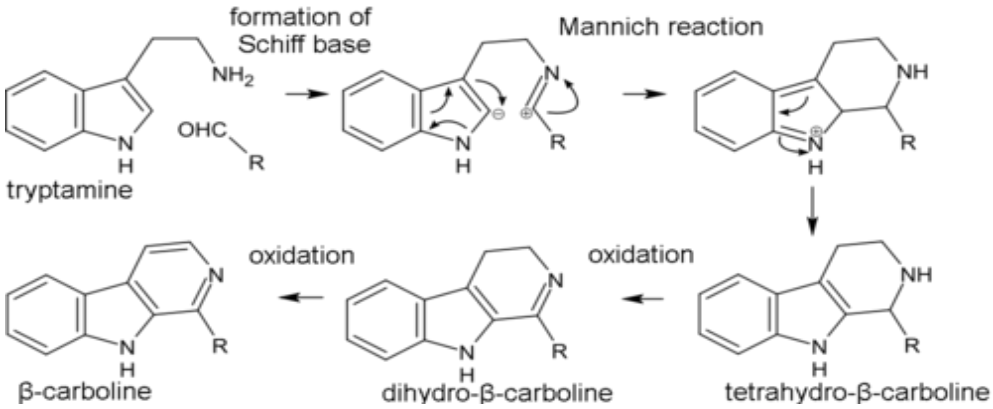

Fig. 4: Semisynthesis of β-carboline

Monoterpene indole alkaloids

(*S*)-strictosidine is the main intermediate for production of monoterpene indole alkaloids (MIAs) and it is generated by condensation process (tryptamine is condensed with monoterpene secologanin). Reserpine is available in market and it is a natural product, extracted from *Rauwolfia vomitoria* in order to produce it on commertial level The biosynthetic pathway for the production of reserpine is yet to be clarified as reserpine has (*R*) stereochemistry at the C3 position, this indicates that precursor for reserpine is (*R*)-strictosidine rather than (*S*)-strictosidine. Anhydrovinblastine is enzymatically or chemically obtained from plant-extracted vindoline and catharanthine in order to semisynthesize vinorelbine . In the result of condensation of vindoline and catharanthine many monoterpene indole alkaloids other than vinorelbine also formed and these alkaloids include vincristine and vinblastine, which are natural products.[45] Production of complex morphinan-derived alkaloids in microbes is now achievable. As biosynthetic pathways for MIAs have not been reveled enough so, production of bisindole MIAs in engineered microbes is lagging behind. However, bisindole MIA production in microbes has been limited up to strictosidine which is an early intermediate, while later intermediates for example tabersonine get converted into vindoline.[46] Limited knowledge of biosynthetic pathways leads to the unavailability of microbes to produce strictosidine while knowledge about some enzymes is available. Strictosidine-β-glucosidase involve in the conversion of Strictosidine into some common intermediates, which ultimately converts to cathenamine *in vitro*. Some enzymes involve in the conversion of tabernosine into vindoline, while α-3,4-anhydrovinblastine synthase produces anhydrovinblastine by dimerization of catharanthine and vindoline. If biosemisynthetic pathways for MIAs production have been illuminated, manufacturing of important intermediates can be possible by using

engineered microbes in order to produce natural as well as modified MIAs.[18]

Pyrroloquinoline alkaloids

(*S*)-strictosidine rearranged in different pattern that results in pyrroloquinoline alkaloids production including the natural products and topoisomerase I inhibitors, camptothecin. From these camptothecin drugs only wo camptothecin derivatives are standardized on market level, these two drugs are topotecan and the anticancer agents irinotecan, while clinical trials or development of other three derivatives are under consideration.[47] On commercial level, plant-extracted camptothecin are used to semisynthesize topotecan and irinotecan**.** *Nothapodytes foetida* and *Camptotheca acuminate* trees are destroyed because plant-extracted camptothecin (600 kg/year) is not enough to meet the requirement for synthesis of camptothecin derivatives (3,000 kg/year)**.** Camptothecin is produced by modification of topotecan and irinotecan at the 10′ position, which is resulted from bipiperidine moiety through ester bond and addition of hydroxyl group, this leads to semisynthesis of 10-hydroxycamptothecin intermediate. Camptothecin derivatives are semisynthesized with production of 10-hydroxycamptothecin by using microbes. Indeed, by using mammalian, plant, bacterial and fungal enzymes, expressed in *S. cerevisiae*, it becomes possible to generate modified MIAs (such as 10-hydroxystrictosidine). 10-hydroxylated MIAs have been produced in *Catharanthus roseus* hairy-root culture which fed with serotonin.[48] However enzymes downstream in the camptothecin pathway would accept 10-hydroxystrictosidine to produce 10-hydroxycamptothecin in microbes as well.

THERAPEUTIC APPLICATIONS

Ergot alkaloids have major effects on vascular smooth muscles but these effects depend upon the type of that particular muscle, such as ergotamine tighten human blood vessels while dihydroergotamine is effective for capacitance vesicles rather than resistance vesicles. Ergot alkaloids drugs are effective for treatment of migraine and severe headache as they decrease inflammation, reverse blood vessel dilation and stimulate serotine.[49] First migraine specific drug is dihydroergotamine, but generally these drugs are prescribed only in rare cases because they also have some side effects including gangrene and ergotism Ergot alkaloids drugs also have some effective response to uterus problems, but it depends upon the hormonal conditions of patient for example pregnancy. Periodic contraction and relaxation occur in case of small doses of ergot alkaloids while expanded contraction and relaxation of uterus take place on high doses.[50] Parkinson's diseases are treated with ergot derived alkaloids by modifying them. These ergot alkaloids drugs include Lisuride (Revanil), Cabergoline (Caberlin), Bromocriptine (Parlodel) and Pergolide (Permax). Parlodel is used as an adjuvant drug with levodopa in order to treat patients with Parkinson's diseases. Now days, Bromocriptine setback the need of levodopa for treatment of parkinson's diseases. Cabergoline is used for treatment of prolactinomas and side effects of Cabergoline are lesser than bromocriptine.[51]

Anti- inflammatory properties are shown by many quinoline, indole and Isoquinoline alkaloids. One of the most important and studied Isoquinoline is berberine. This compound is obtained from *Berberis* and *Coptis* genera.[52] In china, this is used as an excellent herbal drug for the treatment of inflammatory reaction. Berberine is also effective for chronic inflammatory reaction as it is used to inhibit TPA induced mouse ear edema.[53] Other therapeutic activities of berberine include antiulcer, expansion of blood vessels, antibacterial, sedation, antidiabetes, hepatoprotective, neuroprotective, protection of myocardial ischemia-reperfusion injury and inhibition of platelet aggregation. Arrhythmia, diabetes, diarrhea and neurasthenia have also been treated with berberine.[54] Berberines also have an ability to treat cancer as these interfere with several characteristic of tumergenisis. Warifteine is a bisbenzylisoquinoline alkaloid which is isolated from *Cissampelos sympodialis* and has major importance for therapeutic use. Warifteine is very valuable for treating the allergic and inflammatory reactions, as it inhibits eotaxin, eosinophil recruitment, leukotriene and cisteinyl production in the lungs and pleural cavities of allergic mice, as well as production of nitric oxide mediators are also inhibited by Warifteine. Oxymatrine and matrine, types of quinolizidine alkaloids, are extracted from *Sophora subprostrata* and used in Chinese medicines as these demonstrated antioxidant activity, inhibition of cyclooxygenase *in vitro*.[55]

From piperidine alkaloids, piperine is the most important drugs that are isolated from *Piper longum* and *Piper nigrum*. This drug revealed hypolipidemic, tumor inhibitory, antidiarrheal, antimutagenic, antioxidant, antiinflammatory, anticonvulsant and bile secretory activities. Role of piperine in central nervous system as an antidepressant is well known. Tumor development in mice, transplanted with sarcoma 180 cells, is inhibited by piperine. Antibacterial avtivity against *Mycobacterium tuberculosis* H37R is shown by phomoenamide. Indole alkaloids show anticancer activities. Two important indole alkaloids are vincristine[56] and Vinblastine.[57] These compounds demonstrated IC50 values, 3.14-86.95μM, against cholangiocarcinoma cell lines and 2.54-21.29μM against human breast cancer cell line. Among FDA-approved drugs, phenylethylamine and tyramine derivatives are involved in various treatments methods, such as formoterol is used to treat asthama; epinephrine which is an important alkaloid drug as it is used for treating anaphylaxis reaction in emergency case, and phenelzine is used for treatment of depression. Four tetrahydroisoquinoline-derived drugs have been approved by FDA and have major pharmaceutical application. For example, soft tissue sarcomas are treated

with trabectedin while Huntington's disease symptoms are treated with tetrabenazine, praziquantel is an antihelmitic drug and overactive bladder is treated with solifenacin. Alzheimer's disease is treated with galantamine which is the only amaryllidaceae-based pharmaceutical, approved by FDA. Among phenylethylisoquinoline-based pharmaceutical, colchicines, isolated from *Gloriosa superb* on commercial level, is approved by FDA and used to treat acute gout. Two MIA-derived quinolines approved on the market, one is quinidine which used to treat arrhythmia and diastereomers quinine that used to treat malaria.[58]

Evodiamine is one of an important quinolone alkaloid which is extracted and purified from *Evodia rutaecarpa*, a Chinese herb. This drug is effective for treating obesity, anxiety, inflammatory and allergic reactions. Evodiamine also have anticancer properties as they inhibit metastasis, angiogenesis and invasion in range of cancer cell lines and stimulate the apoptosis. Evodiamine is less toxic than other compounds for normal human cells such as peripheral blood mononuclear cells. Sanguinarine is isolated from *Chelidonium majus* L and *Sanguinaria canadensis* L, which are the member of *Papaveracea* family. And these drugs belong to benzophenanthridine alkaloid group. They possess antifungal, anti-inflammatory, antibacterial, antischistosomal and antiplatelet properties. Sanguinarine also have anticancer properties but their effect is much less than 10 micromoles. It has also been recommended that sanguinarine may be developed as an agent for the administration of situation obtained by ultraviolet exposure such as skin cancer.[59]

Tetrandrine is a bisbenzylisoquinoline alkaloid and get from the root of *Stephania tetrandra*. Tetrandrine revealed a broad range of pharmacological activities, including antihepatofibrogenetic, antiportal hypertension, neuroprotective, antiinflammatory, antiarrhythmic, Immunomodulating and anticancer activities. Usually, its anticancer effectiveness is in the micromolar concentrations. Different stages of cell cycle arrest are induced by tetrandrine but it depends on cancer cell types. It also provoked apoptosis in many human cancer cells, including hepatoma, bladder, lung, leukemia and colon. Matrine is a major alkaloid found in many *Sophora* plants, including *Sophora flavescens* Ait. It shows a wide range of pharmacological possessions such as antiinflammatory, antibacterial, cardioprotective antiviral, hepatoprotective, antiasthmatic, diuretic, antiarrhythmic, nephroprotective, antiobesity, anticancer and choleretic effects.[60] It has been used for treatment of malignant pleural effusion, enteritis, bacillary dysentery and so forth in China and the anticancer effects have also been extensively studied.[61] Although the required concentration of matrine to hinder cancer cell proliferation is somewhat high (i.e., at millimolar level) it has no significant effects on the viability of normal cells.[62]

Aside from the aforementioned alkaloids, other alkaloids such as lycorine isolated from *Lycoris*, chelerythrine isolated from *Toddalia asiatica* (L.) Lam, chelidonine isolated from *Chelidoniummajus* L, solanine isolated from *Solanum tuberosum*, fagaronine isolated from *Fagara zanthoxyloides* Lam, nitidine chloride isolated from *Zanthoxylum nitidum* (Roxb.) DC, trigonelline isolated from *trigonella foenum-graecum*and and sophocarpine isolated from *Sophora alopecuroides* L, also have anticancer potentials with diversiform mechanisms. However, reports on the anticancer activities and underlying method of actions of these compounds are inadequate. Although alkaloids drugs have various therapeutic usage but they also have some side effects. For example, the most common side effects of berberine include skin allergies, anaphylaxis and constipation. Piperine stimulated Immunotoxicity, reproductive toxicity and neurotoxicity while sanguinarine can induce embryonic toxicity and hepatotoxicity.[63] Therefore, alkaloids isolated from natural herbs are not always safe. The routes of administration, treatment procedures and dosages are very important. The toxicities of these compounds can be reduced by chemical transformation and the application of new drug delivery systems.

CONCLUSION

The production of alkaloids drugs by plants has some limitations which are overcome with microbial transformation process. Microbial production of modified plant alkaloids has potential to increase the production of FDA approved drugs. Endophytic fungi are also major source of alkaloids pharmaceutical products that has medical as well as agricultural applications though production from these is somehow low. Alkaloids drugs are one of important pharmaceutical product and have effective therapeutic properties such as anticancer, anti-inflammatory, anti allergic, antiobesity, antianxiety and so on. Besides, beneficial properties they also have some side affects most commonly hepatotoxicity, skin allergies and embryonic toxicity. However, modification of plant alkaloids leads to the rapid production of FDA approved pharmaceutical products. But there is hell need to work on their microbial production.

REFERNCES

1. Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry, 2003; 64: 3-19.
2. Canter PH, Thomas H, Ernst E. Bringing medicinal plants into cultivation: Opportunities and challenges for biotechnology. Trends Biotechnol, 2005; 23: 180-183.
3. Leonard E, Yan Y, Fower ZL, Li Z. Strain improvement of recombinant Escherichia coli for efficient production of plant flavonoids. Mol. Pharm, 2009; 5: 257-265.
4. Narcross L, Fossati E, Bourgeois L, Dueber JE, Martin VJ. Microbial factories for the production of

benzylisoquinoline alkaloids. Trends Biotechnol, 2016; 34: 228–241.
5. Paddon CJ, Keasling JD. Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nat. Rev. Microbiol, 2014; 12: 355–367.
6. Rathbone DA, Lister DL, Bruce NC. Biotransformation of alkaloids. Alkaloids Chem Biol, 2001; 57: 1-74.
7. Zhao J, Shan T, Mou Y, Zhou L. Plant-derived bioactive compounds produced by endophytic fungi. Mini-Reviews in Medicinal Chemistry, 2011; 11: 159-168.
8. Wang LW, Zhang YL, Lin FC, Hu YZ, Zhang CL. Natural products with antitumor activity from endophytic fungi. Mini-Reviews in Medicinal Chemistry, 2011; 11: 1056-1074.
9. Zhang Y, Han T, Ming Q, Wu L, Rahman K, Qin L. Alkaloids produced by endophytic fungi: a review. Natural product communications, 2012; 77: 963-968.
10. Puri SC, Verma V, Amna T, Qazi GN, Spiteller M. An endophytic fungus from Nothapodytes foetida that produces camptothecin. Journal of Natural Products, 2005; 68: 1717-1719.
11. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nature Reviews Cancer, 2006; 6: 789-802.
12. Kusari S, Zuhlke S, Spiteller M. An endophytic fungus from Camptotheca acuminata that produces camptothecin and analogues. Journal of Natural Products, 2009; 72: 2-7.
13. Rehman S, Shawl AS, Verma V, Kour A, Athar M, Andrabi R, Sultan P, Qazi GN. An endophytic Neurospora sp. from Nothapodytes foetida producing camptothecin. Prikladnaia biokhimiia I mikrobiologiia, 2008; 44: 225-231.
14. Rehman S, Shawl AS, Kour A, Sultan P, Ahmad K, Khajuria R, Qazi GN. Comparative studies and identification of camptothecin produced by an endophyte at shake flask and bioreactor. Natural Product Research, 2009; 23: 1050-1057.
15. Facchini P. Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. Annu. Rev. Plant Physiol. Plant Mol. Biol, 2001; 52: 29-66.
16. Schäfer H, Wink M. Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. Biotechnology journal, 2009; 4(12): 1684-1703.
17. Kutchan TM, Hampp N, Lottspeich F, Beyreuther K, Zenk MH. The complementary DNA clone for strictosidine synthase from Rauvolfia serpentina DNA sequence determination and expression in Escherichia coli. Febs Lett, 1988; 237: 40-44.
18. Trenchard IJ, Siddiqui MS, Thodey K, Smolke CD. De novo production of the key branch point benzylisoquinoline alkaloid reticuline in yeast. Metab. Eng, 2015; 31: 74-83.
19. Ehrenworth AM, Peralta-Yahya P. Accelerating the semisynthesis of alkaloid-based drugs through metabolic engineering. Nature Chemical Biology, 2017; 13(3): 249-258.
20. Nasreen A, Rueffer M, Zenk MH. Cytochrome P-450-dependent formation of isoandrocymbine from autumnaline in colchicine biosynthesis. Tetrahedr. Lett, 1996; 37: 8161-8164.
21. Ramesha BT, Amna T, Ravikanth G, Gunaga RP, Vasudeva R, Ganeshaiah KN, Uma Shaanker R, Khajuria RK, Puri SC, Qazi GN. Prospecting for camptothecines from Nothapodytes nimmoniana in the Western Ghats, South India: identification of high-yielding sources of camptothecin and new families of camptothecines. Journal of Chromatographic Science, 2008; 46: 362-368.
22. Ilari A, Franceschini S, Bonamore A, Arenghi F. Structural basis of enzymatic (S)-norcoclaurine biosynthesis. J. Biol. Chem, 2009; 284: 897-904.
23. Chang L, Hagel JM, Facchini PJ. Isolation and characterization of O-methyltransferases involved in the biosynthesis of glaucine in Glaucium flavum. Plant Physiol, 2015; 169: 1127-1140.
24. Samanani N, Liscombe DK, Facchini PJ. Molecular cloning and characterization of norcoclaurine synthase, an enzyme catalyzing the first committed step in benzylisoquinoline alkaloid biosynthesis. Plant J, 2004; 40: 302-313.
25. Choi KB, Morishige T, Shita N,Yazaki K, Sato F. Molecular cloning and characterization of coclaurine N methyltransferase from cultured cells of Coptis japonica. J. Biol. Chem, 2002; 277: 830-835.
26. Ikezawa N, Tanaka M, Nagayoshi M, Shinkyo R, Sakaki T, Inouye K, Sato F. Molecular cloning and characterization of CYP719, a methylenedioxy bridge-forming enzyme that belongs to a novel P450 family, from cultured Coptis japonica cells. J. Biol. Chem, 2003; 278: 38557-38565.
27. Ounaroon A, Decker G, Schmidt J, Lottspeich F, Kutchan TM. (R,S)-Reticuline 7-O-methyltransferase and (R,S)-norcoclaurine 6-O-methyltransferase of Papaver somniferum: cDNA cloning and characterization of methyl transfer enzymes of alkaloid biosynthesis in opium poppy. Plant J, 2003; 36: 808-819.
28. Ikezawa N, Iwasa K, Sato F. CYP719A subfamily of cytochrome P450 oxygenases and isoquinoline alkaloid biosynthesis in Eschscholzia californica. Plant Cell Rep, 2009; 28: 123-133.
29. Liscombe DK, Facchini PJ. Molecular cloning and characterization of tetrahydroprotoberberine cis-N-methyltransferase, an enzyme involved in alkaloid biosynthesis in opium poppy. J. Biol.Chem, 2007; 282: 14741-14751.
30. Takeshita N, Fujiwara H, Mimura H, Fitchen JH. Molecular cloning and characterization of S-adenosyl-Lmethionine: Scoulerine-9-O-methyltransferase from cultured cells of Coptis japonica. Plant & Cell Physiol, 1995; 36: 29-36.

31. Ziegler J, Voigtlaender S, Schmidt J, Kramell R. Comparative transcript and alkaloid profiling in Papaver species identifies a short chain dehydrogenase/reductase involved in morphine biosynthesis. Plant J, 2006; 48: 177-192.
32. Unterlinner B, Lenz R, Kutchan TM. Molecular cloning and functional expression of codeinone reductase: The penultimate enzyme in morphine biosynthesis in the opium poppy Papaver somniferum. Plant J, 1999; 18: 465-475.
33. Grothe T, Lenz R, Kutchan TM. Molecular characterization of the salutaridinol 7-O-acetyltransferase involved in morphine biosynthesis in opium poppy Papaver somniferum. J. Biol. Chem, 2001; 276: 30717-30723.
34. Morishige T, Dubouzet E, Choi KB, Yazaki K, Sato F. Molecular cloning of columbamine O-methyltransferase from cultured Coptis japonica cells. Eur. J. Biochem, 2002; 269: 5659-5667.
35. Ikezawa N, Iwasa K, Sato F. Molecular cloning and characterization of methylenedioxy bridge-forming enzymes involved in stylopine biosynthesis in Eschscholzia californica. FEBS J, 2007; 274: 1019-1035.
36. Facchini PJ, Penzes C, Johnson AG, Bull D. Molecular characterization of berberine bridge enzyme genes from opium poppy. Plant Physiol, 1996; 112: 1669-1677.
37. Morishige T, Tsujita T, Yamada Y, Sato F. Molecular characterization of the S-adenosyl-L-methionine:3'-hydroxy- N-methylcoclaurine 4'-O-methyltransferase involved in isoquinoline alkaloid biosynthesis in Coptis japonica. J. Biol. Chem, 2000; 275: 23398-23405.
38. Kraus PFX, Kutchan TM. Molecular cloning and heterologous expression of a cDNA encoding berbamunine synthase, a C-O phenol-coupling cytochrome P450 from the higher plant Berberis stolonifera. Proc. Natl. Acad. Sci. USA, 1995; 92: 2071-2075.
39. Markert A, Steffan N, Ploss K, Hellwig S. Biosynthesis and accumulation of ergoline alkaloids in a mutualistic association between Ipomoea asarifolia (Convolvulaceae) and a Clavicipitalean fungus. Plant. Physiol, 2008; 147: 296-305.
40. Ryan KL, Moore CT, Panaccione DG. Partial reconstruction of the ergot alkaloid pathway by heterologous gene expression in Aspergillus nidulans. Toxins (Basel), 2013; 5: 445-455.
41. Cuevas C, Perez M, Martin MJ, Chicharro JL, Fernández-Rivas C, Flores M, Francesch A, Gallego P, Zarzuelo M, Calle FDL, García J. Synthesis of ecteinascidin ET-743 and phthalascidin Pt-650 from cyanosafracin B. Org. Lett, 2000; 2: 2545–2548.
42. Ruff BM, Bräse S, O'Connor SE. Biocatalytic production of tetrahydroisoquinolines. Tetrahedr. Lett, 2012; 53: 1071-1074.
43. Ehrenworth AM, Sarria S, Peralta-Yahya P. Pterin-dependent mono-oxidation for the microbial synthesis of a modified monoterpene indole alkaloid. ACS Synth. Biol, 2015; 4: 1295-1307.
44. Baumann M, Baxendale IR, Ley SV, Nikbin N. An overview of the key routes to the best selling 5-membered ring heterocyclic pharmaceuticals. Beilstein J. Org. Chem, 2011; 7: 442-495.
45. O'Connor SE, Maresh JJ. Chemistry and biology of monoterpene indole alkaloid biosynthesis. Nat. Prod. Rep, 2006; 23: 532-547.
46. Qu Y, Easson ML, Froese J, Simionescu R, Hudlicky T, De Luca V. Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast. Proc. Natl. Acad. Sci. USA, 2015; 112: 6224-6229.
47. Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolse J. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res, 2006; 34: 668-672.
48. McCoy E, O'Connor SE. Directed biosynthesis of alkaloid analogs in the medicinal plant Catharanthus roseus. J. Am. Chem. Soc, 2006; 128: 14276–14277.
49. Sharma N, Sharma VK, Manikyam HK, Krishna AB. Ergot Alkaloids: A Review on Therapeutic Applications. European Journal of Medicinal Plants, 2016; 14(3): 1-17.
50. Tfelt-Hansen PC, Koehler PJ. History of the use of ergotamine and dihydroergotamine in migraine from 1906 and onward. Cephalalgia, 2008; 28: 877-886.
51. Colao A, Sarno AD, Pivonello R, Somma CD, Lombardi G. Dopamine receptor agonists for treating prolactinomas. Expert Opin Investig Drugs, 2002; 11: 787-800.
52. Yoo KY, Hwang IK, Kim JD, Kang IJ, Park J, Yi JS, Kim JK, Bae YS, Won MH. Anti-inflammatory effect of the ethanol extract of Berberis koreana in a gerbil model of cerebral ischemia/reperfusion. Phytother. Res, 2008; 22: 1527-1532.
53. Souto AL, Tavares JF, da Silva MS, Diniz MDFFM, de Athayde-Filho PF, Barbosa Filho JM. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. Molecules, 2011; 16(10): 8515-8534.
54. Kulkarni SK, Dhir A. Berberine: a plant alkaloid with therapeutic potential for central nervous system disorders. Phytotherapy Research, 2010; 24(3): 317-324.
55. Ao C, Araki N, Tawata S. Cyclooxygenase inhibitory compounds with antioxidant activities from Sophora subprostrata. Asian J. Chem, 2009; 21: 745-754.
56. Wang F, Xu M, Li Q, Sattler I, Lin W. p-Aminoacetophenonic acids produced by a mangrove endophyte Streptomyces sp. (strain HK10552). Molecules, 2010; 15: 2782-2790.
57. Zhang Y, Li XM, Wang CY, Wang BG. A new naphthoquinoneimine derivative from the marine algal-derived endophytic fungus Aspergillus niger EN-13. Chinese Chemical Letters, 2007; 18: 951-953.

58. Lu JJ, Bao JL, Chen XP, Huang M, Wang YT. Alkaloids isolated from natural herbs as the anticancer agents. Evidence-Based Complementary and Alternative Medicine, 2012; 2012: 12.
59. Ahsan H, Reagan-Shaw S, Eggert DM, Tan TC, Afaq F, Mukhtar H, Ahmad N. Protective effect of sanguinarine on ultraviolet B-mediated damages in SKH-1 hairless mouse skin: implications for prevention of skin cancer. Photochemistry and Photobiology, 2007; 83(4): 986-993.
60. Xing Y, Yan F, Liu Y, Liu Y, ZhaoY. Matrine inhibits 3T3-L1 preadipocyte differentiation associated with suppression of ERK1/2 phosphorylation. Biochemical and Biophysical Research Communications, 2010; 396(3): 691-695.
61. Liang CZ, Zhang JK, Shi Z, Liu B, Shen CQ, Tao HM. Matrine induces caspase-dependent apoptosis in human osteosarcoma cells in vitro and in vivo through the upregulation of Bax and Fas/FasL and downregulation of Bcl-2. Cancer Chemotherapy and Pharmacology, 2011; 69(2): 317-331.
62. Liu T, Song Y, Chen H, Pan S, Sun X. Matrine inhibits proliferation and induces apoptosis of pancreatic cancer cells in vitro and in vivo. Biological and Pharmaceutical Bulletin, 2010; 33(10): 1740-1745.
63. Choy CS, Cheah KP, Chiou HY, Li JS, Liu YH, Yong SF, Chiu WT, Liao JW, Hu CM. Induction of hepatotoxicity by sanguinarine is associated with oxidation of protein thiols and disturbance of mitochondrial respiration. Journal of Applied Toxicology, 2008; 28(8): 945-956.