Biodiversity and natural product drug discovery

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Abstract: The threat to biodiversity through the destruction of terrestrial and marine ecosystems coupled with the urgent need to find novel, new chemotherapeutic agents as well as active chemotypes as leads for effective drug development, make natural products research in drug discovery and development a top priority. The paper will highlight the research of selected natural products aiming at the discovery of therapeutic agents from Thai plants. Attention will be focussed on selected plants that possess cytotoxic properties.

INTRODUCTION

Biodiversity - the diversity of living forms - has lately attracted a great deal of interest and concern since biological resources constitute an asset with a great deal of immediate as well as potential benefit for the quality of life. Ironically, just as we begin to recognize some of the potential benefits that might accrue from our having a large number of species, we are also coming to a realization that there is a current decline in the number of species and that this may have catastrophic consequences (1). This decline in biodiversity is largely the result of human activities such as drastic transformation of natural landscapes or deforestation. These phenomena pose a serious threat to sustainable development since species diversity may well be our planet's most important and irreplaceable resource. Once depleted, species regeneration, if at all possible, might take 5 to 10 million years. Its loss would thus have profound negative effects on the overall quality of life on our planet and on our potential development. The consequences of a reduction in biodiversity through loss of species constitute a serious threat to human survival. The loss of species could reduce the availability of natural products used as raw materials for manufacturing and industry. It could also diminish the future availability of new genetic resources and wild germ plasm essential for breeding crop varieties with higher productivity and with greater resistance to insects, diseases, and adverse climatic conditions. In medical science, the loss of species could reduce the opportunity for treatment of diseases through the loss of medical models and new medicines as a result of reduction of the availability of natural products which have potential medicinal properties. Animals and human beings share certain similarities as well as differences in a number of biochemical processes and
mechanisms. It is through the knowledge and research findings obtained from studies in animals that many physiological and biochemical mechanisms are unravelled, particularly those underlying the etiology of diseases that lead to development of therapeutic methods and discovery of agents currently employed.

Some animal species that are currently at risk have been shown to be valuable medical models offering windows for greater understanding of human physiology and biochemistry which may lead to successful treatments of diseases that are at present incurable. An example of medical model developed from animals species that would appear to be far apart in the biosystem is highlighted by research on the active constituents of frog toxins. The research undertaken by John Daly of the National Institutes of Health, USA, and colleagues since the early 1970's deserves special mention (2). Certain types of frogs found in tropical rain forests in Central and South America produce a wide range of biologically active alkaloids, which make the dart-poison frogs (Dendrobatidae) an extremely important medical model in our understanding of cross-species biochemistry. Some of these alkaloids are important scientific tools in the study of the basic unit of membrane function and neurophysiological research. The study of the mechanism of action of some of these frog toxins may provide clues for the production of new therapeutic agents for treatment of diseases such as Alzheimer's disease and other neurological disorders. The remarkably potent analgesic epibatidine was also discovered during these investigations. Unfortunately, continued deforestation will likely result in the extinction of the dart-poison frog because of the destruction of their habitat and the resulting loss of a possible tool for studying neurological disorders and other diseases.

The loss of other species could have immediate negative effects on the use of medicines derived from these endangered species. The rapid dying out of the periwinkle plant illustrates the negative result of deforestation. Both vinblastine and vincristine (3), derivatives of the periwinkle plant, have proved to be effective against tumor growth. Vinblastine has been shown to be a very effective treatment for Hodgkin's disease, and has also been used to treat breast cancer, Kaposi's sarcoma, and other diseases. Vincristine is well known for its 90% success rate in treating different types of childhood leukemia. Extinction of the periwinkle plants as a result of deforestation would thus result in a loss of treatments which have proven effective in treating certain illnesses. Throughout the ages, humans have exploited the cornucopia of nature as a source of medicines for the treatment of a variety of diseases. Plants have formed the basis for traditional medicine for thousand of years.

It is clear that demand for drugs, disposable consumer products, biological agents and insecticides will continue to increase for the foreseeable future. But unless we take specific action to protect and develop our environment under sustainable conditions, the window of opportunity for the discovery of new medicinal and biological agents will be shut forever.

**IMPORTANCE OF NATURAL PRODUCTS IN DRUG DEVELOPMENT**

In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine, plants, and micro-organisms. It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovering of potential new drugs and biological entities. However, only 5-15% of the world's approximately 250,000 flowering plants have as yet been analysed for their possible medicinal uses (4). The most alarming
cause for concern is that, by the turn of this century, it is expected that some 25,000 species of plants will have ceased to exist. This represents about five plant species a day between now and the year 2000.

Moreover, in developing countries, medicinal plants continue to be the main source of medication. In China alone, 7,295 plant species are utilized as medicinal agents. The World Health Organization has estimates that for some 3.4 billion people in the developing world, plants represent the primary source of medicine. This represents about 88% of the world's inhabitants who rely mainly on traditional medicine for their primary health care (5). It is thus a matter of utmost concern to public health and indeed to human life that urgent action is taken to prevent further diminution of actual and potential availability of medicinal and biological agents. Natural remedies that, although undocumented, may have been used for many thousands of years by the human race must be appropriately catalogued to ensure that vital ethnomedical information is not lost for ever. Farnsworth et. al. reported that at least 119 compounds derived from 90 plants species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine (5). The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives (6).

NATURAL PRODUCTS AS ANTICANCER AGENTS

Apart from being an excellent source of anti-infectious drugs (8,13,14) plants are also good source of anticancer agents (7-12). National Cancer Institute (NCI), USA has launched an extensive program for the development of natural products for the treatment of various forms of cancer. Many clinically useful drugs have been discovered from various plants. These include vinblastine and vincristine from Catharanthiis roseus and the semisynthetic, etoposide, as well as the recently discovered taxol and the semi synthetic taxotere. Taxol(paclitaxel) has been isolated from the bark of the Pacific or American yew tree, Taxus brevifolia. The discovery of taxol has indeed heightened the interest in plant-derived anticancer drug.

The addition of taxol to the list of anticancer drug is testimony to the synergism of broadly based contributions from multidisciplinary scientific endeavour. The isolation and structure elucidation led the way to the pharmacological and toxicological testings. The finding of baccatin III from other phytochemical sources coupled with the synthetic organic chemistry make the drug available for human trials. The finding that taxoids act through the stabilization of microtubules has led to the search of new agents that function by a comparable mechanism. Towards this end, new compounds have been discovered. Epothilones (15) are a new class of macrocyclic natural products which were first isolated from myxobacteria (16). Epothilones are more potent than taxol in some cell lines and they hold great promise for further investigation. In addition to the above mentioned clinically approved drugs and promising drug candidates, some other plant-derived compounds show a great deal of promise for future use as anticancer agents. Camptothecin (17) was originally isolated from the Chinese tree Camptotheca acuminata and a number of camptothecin analogues (18) are currently being developed as anticancer agents. Camptothecin was established as having in vivo activity against the murine leukemia and rat Walker carcinosarcoma 256 models. While early clinical trials on the parent alkaloid and camptothecin sodium were not particularly successful due to toxicity.
problems, interest in camptothecin intensified once it was discovered that it exhibits a novel mechanism of action by inhibiting the enzyme DNA topoisomerase I. Accordingly, a number of camptothecin analogues have been developed in an attempt to reduce toxicity, optimize efficacy, and improve water solubility without opening the lactone ring present in the parent molecule. Topotecan is one of the camptothecin analogue which is under clinical trial. Another plant derived alkaloid which is also under clinical trial is homoharringtonine (19,20), a cephalotaxine alkaloid. The compound was originally isolated from *Cephalotaxus harringtonia*, it shows antineoplastic activity, especially against murine lymphocytic leukemias. It was found to be more active than vincristine against mouse leukemias and melanomas.

Apart from our study of *Phyllanthus amarus* (21), we have also investigated *Gloriosa superba* Linn. for anticancer activity. *Gloriosa superba* Linn. is known in Thai as “Dong Dueng” or “Dao Dueng”, a climber plant in the family "Colchicaceae", which is widely distributed in the tropical part of Asia and Africa, with many varieties presented in Thailand.

The active principle of *Gloriosa superba* is colchicine which has long been used for the treatment of arthritis. From the dried tuber of *Gloriosa superba*, four tropolone alkaloids were isolated and identified as colchicine, lumicolchicine, 3-demethyl-N-formyl-N-deacetylcolchicine and 3-demethylcolchicine (22).

![Chemical structures](image)

The structures were elucidated using various spectroscopic techniques including UV, IR, MS, and one- and two dimensional NMR.

The biological activity testings of various extracts of *Gloriosa superba* for cytotoxicity were carried out using the P 388 cell line, with 5-fluoro-uracil (5-FU) as the positive control. The ED50 of the different "Gloriosa" extracts are shown in the table.

Colchicine exhibits very low ED50 value suggesting the potent cytotoxicity of the compound. The chloroform, methanol and petroleum ether extracts of *Gloriosa superba* also show very low ED50 value which could result from the presence of colchicine in these extracts.
It is interesting to note that the ED\textsubscript{50} value of 3-demethyl-\textit{N}-formyl-\textit{N}-deacetyl-colchicine is very close in value to the ED\textsubscript{50} of 5-FU which is a widely used anticancer agent, suggesting the strong cytotoxic potency of this compound. In this particular test, \textit{\beta}-lumicolchicine was found to be inactive, suggesting that the tropolone ring is crucial for the anticancer activity.

Methanol extract was tested against other cancer cell lines, such as KB-3, KB-V-1, BCA-1, HT-1080, LUC-1, MEL-2, COL-2, A-431, LNCaP, Lul and ZR-75-1 cell lines. Some relevant results are shown in the table.

The results are expressed as the percent growth of the cells treated with the extracts at the concentration of 20 microgram per ml. The extracts exhibited a very potent activity against the drug-sensitive KB-3 cell line, however, it did not mediate a cytotoxic response in the multidrug-resistance KB-V1 cell line, in the presence or the absence of vinblastine. In addition, the extract showed potent activity against the human lung cancer, prostate cancer and breast cancer cell lines.

Another cancer cell line of our interest is the cholangiocarcinoma cell lines. Cholangiocarcinoma (23), a form of bile duct cancer, is a rare type of cancer in the western world but it is highly prevalent in Thailand and in many other Asian countries in our geographical region. The cause of the disease is believed to be associated with infestation of \textit{Opisthorchis viverrini}(O.V.) or liver fluke and exposure to a chemical carcinogen in food or in the environment, presumably dimethylnitrosamine (DMN).

We have been interested in the evaluation of the effectiveness of some new anticancer agents against cholangiocarcinoma. This process was carried out by the \textit{in vitro} testing of our established cholangiocarcinoma (HuCCA-1) cell line (24). Some colchicine derivatives have been subjected to cytotoxicity testing, using microculture protein assay and the results are as shown.
The ED$_{50}$ values (µg/ml) against KB and HuCCA-1 cell lines

<table>
<thead>
<tr>
<th>Gloriosa superba</th>
<th>KB</th>
<th>HuCCA-1</th>
</tr>
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<tbody>
<tr>
<td>Methanol extract</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>3-Demethyl-N-formylcolchicine</td>
<td>0.03125</td>
<td>0.0625</td>
</tr>
<tr>
<td>3-Demethyl-N-formyl-N-deacetylcolchicine</td>
<td>0.03125</td>
<td>0.0625</td>
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HuCCA-1 = Human cholangiocarcinoma cell line

The ED$_{50}$ values for the cholangiocarcinoma cell line for the methanol extract was found to be 2.5 micrograms per ml, while both the ED$_{50}$ of 3-demethyl-N-formylcolchicine and 3-demethyl-N-formyl-N-deacetylcolchicine was found to be 0.0625 microgram per ml, in contrast with the ED$_{50}$ value of about 0.5 microgram per ml of colchicine. These values were approximately two times higher than the ED$_{50}$ values for the KB cell line, in all tests conducted. These results showed that the cholangiocarcinoma cell line is highly susceptible to the derivatives of the tropolone alkaloids, at least when testing in vitro. Whether or not these agents will be effective in vivo remains to be determined in further experiments.

We have recently investigated another plant called Derris reticulata locally known as Cha-aim thai (25). Cha-aim thai is a medicinal plant of Thailand used for the relief of thirst and as an expectorant. Our studies led to the isolation of two new pyranoflavanone compounds. Lupinifolin (I), a known flavanone was isolated as the major constituent of this plant.

Detailed analysis of NMR spectra through COSY, NOSEY, APT, HETCOR and selective INEPT confirmed the structure of lupinifolin (I) as the flavanone derivative as shown. The first unknown isolated, named epoxyepinifolin (II), was proved to be the 2,3 epoxide of lupinifolin by spectroscopic methods. The structure of the epoxide was further confirmed by successful epoxidation of lupinifolin with magnesium monoperoxyphthalate hexahydrate (MMPP). The other new compound isolated was named dereticulatin (III) and the structure was found to be hydroxy derivatives through the analysis of the NMR spectra of the corresponding triacetate.
With regard to the biogenetic relationship of these flavanones, dereticulatin could be considered to be derived from epoxylupinifolin by the opening of the epoxide ring. Epoxylupinifolin could be formed from oxidation of lupinifolin. Alternatively, it is appealing to speculate the Ene reaction of the double bond in the lupinifolin to give the hydroperoxide intermediate which could then react with lupinifolin again to give directly the epoxylupinifolin and dereticulin.

We have also carried out the in vitro bioassay evaluation of lupinifolin, epoxylupinifolin and dereticulin triacetate. Each of the compounds inhibited the P-388 cell line at 0.4-0.5 microgramme per ml, but were inactive against the KB cell line.

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REFERENCES