

Endophytes and the microbial genetics of traditional medicines



Kristin I Miller^{1,2}, *Shane D Ingrey*²,
*Alfonso Alvin*², *Man Yuen Daniel*
*Sze*³, *Basil D Roufogalis*¹ & *Brett A*
*Neilan*²

¹ Faculty of Pharmacy, University of Sydney
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² School of Biotechnology and Biomolecular
Sciences, University of New South Wales
NSW 2052

³ Department of Health Technology and
Informatics, The Hong Kong Polytechnic
University, Hung Hom Kowloon Hong Kong

Corresponding author
Brett Neilan

School of Biotechnology and
Biomolecular Sciences
University of New South Wales
NSW 2052

Tel (02) 9385 3235
Fax (02) 9385 1483

Email b.neilan@unsw.edu.au

Traditional medicine continues to play an essential role in the healthcare systems of many cultures. In some Asian and African countries up to 80% of the population depend on these ancient and culturally based medicinal practices for their primary healthcare needs¹ (Figure 1). Plants and their derived natural products are frequently employed as traditional medicine and such plants are viewed as attractive targets for the discovery of novel therapeutic agents in natural product investigations. A variety of useful drugs has been discovered following the investigation of traditional herbs², such as morphine (analgesic), digitoxin (cardiotonic) and ephedrine (sympathomimetic). These ethnopharmacology approaches to drug discovery are based on the premise that plants used as traditional medicines have shown some form of bioactivity and have the increased likelihood of containing bioactive compounds in comparison to plants selected at random. Three systems of traditional medicine that are relevant to Australian drug discovery researchers include the Chinese, Australian Aboriginal and Indonesian systems.

Tradition medicines

Traditional Chinese medicine (TCM) is a major system of traditional medicine with origins traced to the dawn of civilisation in China. The use of plants in TCM was developed in an empirical manner through observation of the effects TCMS produced on specific ailments and certain parts of the body³. TCMS are frequently the subject of ethnopharmacology studies. The value of utilising TCM plants for the discovery of new bioactive compounds is illustrated by the discovery of the anticancer compound indirubin. Here, a well-known traditional herbal mixture named Dangui Longhui Wan was chosen for investigation due to its modern application in the treatment of chronic myelogenous leukaemia and its traditional “purging of liver fire” properties⁴. *Indigofera tinctoria* was identified

as the bioactive herb and from this the bioactive compound indirubin was isolated. Indirubin shows anticancer activity by inhibiting cyclin-dependent kinases and blocking the cell cycle⁵. It is, therefore, probable that it is responsible for the anticancer activity observed by the Dangui Longhui Wan formula.

Australian Aborigines are the original inhabitants of Australia and have the oldest surviving and continuing culture in the world today. There were an estimated 300 tribes Australia-wide, with a vast knowledge of Australia’s unique flora and their medicinal use⁶. The most famous and commercial example of traditional Australian medicine is tea-tree oil. The main source of extracted tea-tree oil is from *Melaleuca alternifolia* and comprises approximately 100 terpenes, although the main antimicrobial constituent has been identified as terpin-4-ol^{7,8}. Tea-tree oil has a range of activities: antibacterial, antifungal, antiviral and anti-acne, to name a few. Other recently studied plants from Australia include Red Poverty Bush (*Eremophila duttonii*) which exhibits broad range antibacterial activity with the active compounds being two serrulatane diterpenes^{9,10}.

Australia’s close neighbour Indonesia also has a history of herbal medicine and is a biodiversity hot spot, with its complex geological history, the existence of many islands that drive the development of endemic species and a tropical climate which supports the growth of many plants¹¹. Approximately 10% of these plants are believed to possess some medicinal characteristics¹². While local Indonesian tribes have utilised the medicinal properties of plants for centuries, the active constituents have not been thoroughly studied. The herb, commonly known as sambiloto, *Andrographis paniculata*, is traditionally used in the treatment of the common sore throat, hypertension, bronchitis, pulmonary tuberculosis and diabetes. Analysis of the ethanol extract from the herb revealed the presence of andrographolides, which are powerful bioactive compounds that possess anti-hepatotoxic, immunostimulatory, and anticancer activities^{13,14}. Similarly, the

fruit from *Brucea javanica* (local name: buah makasar) has been used in the treatment of dysentery, malaria and various forms of cancer¹⁵. The quassinoids, bruceantin and bruceine have been identified as the biologically active compounds in *Brucea javanica* extracts^{16,17}.

Most small molecules isolated as natural products are secondary metabolites, compounds involved in the relationship of the organism with the environment or with other organisms. They differ from primary metabolites as they are not required for normal growth, development or reproduction of the organism¹⁸. Production of secondary metabolites can be the result of genetic, developmental and environmental factors¹⁸. They also feature diverse chemical structures and have often evolved to possess biological activities with roles as defensive compounds against competitors/parasites/predators, growth and reproduction facilitators, or as cell-signalling compounds¹⁹.

Endophytes

There is mounting evidence that many bioactive compounds isolated from plants, as well as marine and terrestrial invertebrates, are actually metabolites produced by symbiotic microorganisms²⁰. A significant niche for symbiotic microbes exists within plants. Endophytes are microorganisms, bacteria or fungi, that reside inside the healthy tissues of the host plant, and are transiently symptomless²¹. All parts of plants are frequently

colonised by unknown numbers of microbial endophytes²²⁻²⁴ and their populations can be influenced by host affinities towards the endophytes or by external environmental conditions²⁵.

Endophytes have the ability to produce a range of secondary metabolites, providing researchers with numerous leads for compounds of pharmaceutical significance and possible development as new drugs²⁶. Taxol, camptothecin and podophylotoxin are important pharmaceutical natural products produced by plants; however, recently their production by endophytes has also been reported²⁷⁻²⁹. This metabolic diversity is accomplished by unique genetic systems and is hypothesised to have developed as a result of endophytes inhabiting environmental niches provided by plants in diverse terrestrial and aquatic habitats^{21,30}. In particular, endophytes of medicinal plants are of interest in drug discovery studies as they may produce compounds which contribute to the plant's bioactivity (Table 1³¹⁻³⁷). In a recent study, three Chinese pharmaceutical plants that produced anticancer and antifungal compounds were investigated for their endophytic fungi³⁸. More than half of the fungi isolated in the study displayed growth inhibition activities towards at least one of the pathogenic test fungi.

The endophytes of Australian medicinal plants have also been the subject of investigation. In one study, a plant used for treating infections and wound healing, called Snakevine (*Kennedia nigricans*) was investigated as a source of endophytes. One



Figure 1. Traditional medicine plants of many cultures continue to have significance in medicine today. St John's wort (*Hypericum sp.*) is a common herbal treatment for depression and it has a history of use in Native American **1** and Ancient Greek **4** herbal medicine. Acai palm fruit (*Euterpe oleracea*), used by Brazilian **2** and other South American cultures, contains high level of antioxidants and possesses anticancer properties. African **3** cultures have relied on herbal medicines, such as Devil's claw (*Harpagophytum procumbens*). Traditionally used as an anti-inflammatory, it is now used to relieve pain and inflammation associated with degenerative joint diseases. The Indian **5**, Chinese **6**, Korean **7**, Japanese **8** and Indonesian **9** herbal medicine systems all make use of the immune-stimulating herb *Andrographis paniculata*. Australian Aborigines **10** utilise Australian native plants including *Acacia sp.*, which have shown anti-inflammatory and antiviral properties.

isolated strain, *Streptomyces* sp. (NRRL 30562), produced a novel class of antibiotics, munumbicins³³. Munumbicins E-4 and E-5 have broad range antibiotic activity as well as antimalarial activity at levels two orders of magnitude higher than chloroquine³⁹. Another endophyte study isolated the strain *Streptomyces* sp. NRRL 30566 from the plant species *Grevillea pteridifolia*. This strain produced two novel antibiotics, kakadumycin A and B, which showed antibacterial activity in the same order as the drug echinomycin, in particular against *Bacillus anthracis*³⁴. In the same study echinomycin and kakadumycin A were tested against the human breast cancer line BT20, exhibiting an IC₅₀ of 6.5 and 4.5 ng/ml, respectively, revealing kakadumycin A as a potential anticancer molecule.

Endophytic fungi isolated from *Brucea javanica* grown in West Java, Indonesia, have been found to produce compounds that possess anticancer activities with IC₅₀ values against a leukaemia cell line ranging from 3.29-15.90 µg/mL¹⁵. Additionally, an endophytic strain of *Muscodor albus* was isolated from a traditional plant used by a Sumatran tribe for the treatment of snakebite infections. The endophyte was shown to produce volatile organic compounds which have both antibacterial and antifungal activity⁴⁰.

Genetic basis of secondary metabolites

Advances in our understanding of the genetics and biosynthesis of microbial secondary metabolites during the last 15 years have reinvigorated the study of natural products for drug discovery⁴¹. Natural products can be divided into several classes based on assembly pathways. It is evident that a plethora of microbial secondary metabolites are polyketides and non-ribosomal peptides, which are biosynthesised by polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS)

systems, respectively. Consequently, identification of PKS and NRPS biosynthetic pathways can be used to evaluate an organism's potential to produce bioactive compounds⁴² and endophytes have been the subject of such investigations⁴³. Genes involved in the production of secondary metabolites appear to be clustered in fungi and bacteria⁴⁴ and genetic screening methods have gained attention because they are rapid, economical and sensitive. These genetic screens can replace previous natural product discovery programs and potentially lead to the sustainable production of bioactive compounds. Increased knowledge of the genetic basis for biosynthetic pathways will also aid in the future manipulation of the final structure and, therefore, activity of these natural products.

Conclusion

Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with immense medicinal and agricultural potential. It has been shown that plants employed as traditional medicines are host to microbes that make these bioactive natural products and their derivatives that may be more bioactive than those of their respective hosts. Further research in this field is required at the molecular level for a better understanding of host–endophyte interactions and secondary metabolism biosynthesis. This will afford the more rapid recognition of the genetic potential of endophytes and facilitate the natural product discovery process. Comprehensive efforts are needed to culture or clone the genomic potential of the endophytes of medicinally important plants. This is a significant global resource, given the number of traditional peoples with history of medicinal herbs. Taken as a whole, endophytes promise to be a significant prospect for scientific discovery in the future.

Table 1. Natural products biosynthesised by endophytic microorganisms from medicinal plants.

Plant	Traditional medicine	Endophyte	Product	Activity	Biosynthetic pathway	Structure	Ref
<i>Cinnamomum zeglamicum</i>	Chinese/ Indian	<i>Muscodor albus</i>	1-butanol, 3-methyl-, acetate	volatile antimicrobial	unknown	ester	31
<i>Ephedra fasciculata</i>	Chinese/ Indian	<i>Cbaetomium cbiversii</i> C5-36-62	Radicalol	cytotoxic	PKS	polyketide	32
<i>Grevillea pteridifolia</i>	Australian Aboriginal	<i>Streptomyces</i> sp. 30566	Kakadumycin A	broad range antibacterial, antifungal, cytotoxic	Unknown	peptide	33
<i>Kenmedia nigriscans</i> (snakevine)	Australian Aboriginal	<i>Streptomyces</i> NRRL 30562	Munumbicin E-4 and E-5	broad range antibacterial, antifungal, antimalarial	Unknown	peptide	34
<i>Scutellaria barcalenis</i>	Chinese	<i>Bacillus amyloiquefaciens</i>	fengycins	antifungal, plants growth promoter	NRPS	lipo-peptide	35
<i>Taxus chinense</i>	Chinese	<i>Fusarium solani</i>	Taxol	anticancer	Unknown	terpene	36
<i>Tripterygium wilfordii</i>	Chinese	<i>Fusarium subglutians</i>	Subglutinol A and B	immuno- suppressive	Unknown	terpene	37

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Biographies

Kristin Miller, Dr Daniel Sze and Professor Basil Roufogalis are members of the Herbal Medicines Research and Education Centre at the University of Sydney, where Professor Roufogalis is executive director of the Centre and Pro Dean of the Faculty of Pharmacy. The Centre covers a broad spectrum of medicinal and natural product chemistry, pharmacological and cell biological sciences and undertakes collaborative research projects in cancer management and diabetes with groups within the University, other national and international universities and research institutes, the herbal medicines industry and the profession.

Professor Brett Neilan is head of the UNSW Cyanobacteria Research Laboratory, deputy director of the Australian Centre for Astrobiology and an ARC Federation Fellow. His research group at UNSW is considered to be one of the world's leaders in the genetics of toxic cyanobacteria. More recently, Professor Neilan has become involved in the search for potentially useful genes occurring in bacteria and fungi of unique environments.

Kristin Miller, Shane Ingrey and Alfonsus Alvin are PhD students with projects investigating the endophytes of medicinal plants used as traditional Chinese, Australian Aboriginal and Indonesian medicines, respectively.