Perspective paper

Ethnobotany/ethnopharmacology and mass bioprospecting: Issues on intellectual property and benefit-sharing

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Abstract

Ethnobotany/ethnopharmacology has contributed to the discovery of many important plant-derived drugs. Field explorations to seek and document indigenous/traditional medical knowledge (IMK/TMK), and/or the biodiversity with which the IMK/TMK is attached, and its conversion into a commercialized product is known as bioprospecting or biodiversity prospecting. When performed in a large-scale operation, the effort is referred to as mass bioprospecting. Experiences from the mass bioprospecting efforts undertaken by the United States National Cancer Institute, the National Cooperative Drug Discovery Groups (NCDDG) and the International Cooperative Biodiversity Groups (ICBG) programs demonstrate that mass bioprospecting is a complex process, involving expertise from diverse areas of human endeavors, but central to it is the Memorandum of Agreement (MOA) that recognizes issues on genetic access, prior informed consent, intellectual property and the sharing of benefits that may arise as a result of the effort. Future mass bioprospecting endeavors must take heed of the lessons learned from past and present experiences in the planning for a successful mass bioprospecting venture.

Keywords: Ethnobotany; Ethnopharmacology; Mass bioprospecting; NCI; NCDDG; UIC ICBG; Intellectual property; Prior informed consent; Indigenous and traditional knowledge; Benefit-sharing

1. Introduction

The definition of the term ethnobotany, originally applied to the study of the utilitarian relationship [relationship that includes use for medicinal purposes] between humans and the plant environment in primitive settings (Harshberger, 1896),
has now evolved into a much broader meaning that covers not only a utilitarian relationship, but also relationships that embrace the symbolic, ecological and cognitive, as well as the human–plant relationship in a modern setting (Schultes and von Reis, 1995; Alexiades, 1996). On the other hand, the more recent term ethnopharmacology has undergone only slight evolution in meaning since its original definition as "a multi-disciplinary area of research, concerned with the observation, description and experimental investigation of indigenous drugs and their biological activities" (Rivier and Bruhn, 1979). The contemporary definition addresses the "interdisciplinary study of the physiological actions of plant, animal and other substances used in indigenous medicines of past and present cultures" (International Society of Ethnopharmacology Constitution, 2005). The breadth of studies embraces "use of plants, fungi, animals, microorganisms and minerals and their biological and pharmacological effects based on the principles established through international convention", as well as "the observation and experimental investigation of the biological activities of plant and animal substances", using ethnopharmacological, ethnobotanical, ethnochemical, pharmacological and toxicological approaches (Journal of Ethnopharmacology, 2005).

Since these two branches of scientific disciplines carry a common denominator, namely the human or cultural component inherent in the word "ethno", ethnobotanical and ethnopharmacological studies normally involve field explorations of indigenous as well as traditional medical knowledge (IMK/TMK), together with the biodiversity component to which such knowledge is attached, documentation and conversion of the knowledge into a product, be it a scholarly paper, a book, photographic images or a tangible product of commercial value. In this process, when the purpose and outcome are commercial in nature, the activity is known today as “bioprospecting”, a concept originally introduced in 1989 for “chemical prospecting” (Eisner, 1989, 1992) and was redefined in 1993 as “biodiversity prospecting” (Reid et al., 1993). The term applies also to the exploration and utilization of the biological diversity itself for commercial purposes, either within the context of IMK/TMK or without.

Twenty-five years ago, when the Journal of Ethnopharmacology was established (Journal of Ethnopharmacology 1 (1) 1979), bioprospecting was considered natural and justified, because the outcome benefits the scientific and general communities, locally and globally. In fact, bioprospecting has been going on for centuries, but only within the past 20 years has this activity gained prominence due to technological advances in pharmaceutical, biotechnological and agricultural sectors. Before this period, little thought was given to the issue of ownership of IMK and TMK and of the distribution of benefits that may arise as the result of the bioprospecting effort. This had been due, in part, to the general attitude at that time that the world’s biodiversity, namely the genetic or biological resources with which IMK/TMK is attached, represents a common heritage of man (FAO, 1983) and so does the IMK/TMK.

2. Recognition of IMK/TMK and “sovereign rights” over genetic resources

The turning point on the formal recognition of ownership of indigenous and traditional knowledge took place in 1988 at the first International Congress of Ethnobiology (ICE) in Belem (Brazil), with the “Declaration of Belem” (http://guallart.dac.uga.edu/ISE/iseBelem.html), which is embodied within the Code of Ethics of the International Society of Ethnobiology (2005) (http://guallart.dac.uga.edu/ISE/iseEthics.html). The Code of Ethics states that "indigenous peoples, traditional societies and local communities have a right to self determination (or local determination for traditional and local communities) and that researchers and associated organizations will acknowledge and respect such rights” (Principle 2) and that they "must be fairly and adequately compensated for their contribution to ethnobiological research activities and outcomes involving their knowledge” (Principle 12). In the context of IMK/TMK, IMK/TMK represents the property of the holders of that knowledge and must be respected, and that compensation to the holders must be provided for the utilization and conversion of such knowledge (bioprospecting) into a tangible product. In the second ICE in 1991, in Kunming, PRC, participants went further to declare that traditional and indigenous knowledge are inventive and intellectual, therefore, worthy of protection in all legal, ethical and professional frameworks (International Society of Ethnobiology Constitution, 2005).

In 1992, the recognition of ownership of traditional knowledge and the biodiversity to which the knowledge is attached, and the sharing of the benefits that may arise as a result of their utilization was enforced in the form of the United Nations Convention on Biodiversity (Secretariat of the Convention on Biological Diversity, 2001, Articles 15.1 and 8.j).

The Belem Declaration, the Kunming Action Plan, the United Nations Convention on Biodiversity and numerous other Declarations of similar nature represent the instruments for States to legislate the utilization and protection of their natural resources that include the biological diversity. One of the earlier biodiversity policy regulations enacted by a country was the Executive Order 247 (EO 47) (1995) introduced by the President of the Republic of the Philippines. Other countries have also enacted legislation or are in the process of regulating their policy. Numerous national and international forums have been convened toward the legislation of a national bioprospecting policy. Central to any policy on bioprospecting are the issues on intellectual property, as it pertains to IMK/TMK, and the benefit-sharing of the process and the outcome that may arise from the activity.

Bioprospecting is performed by a diverse class of people, non-scientists and scientists alike. Although the context of this paper is ethnobotany and ethnopharmacology, the discussion that follows covers bioprospecting activities triggered by the search and utilization of IMK/TMK, as well as by the search and utilization of the component to which IMK/TMK is attached. When the activity of bioprospecting involves a
large-scale effort to search and commercialize IMK/TMK and/or the biological diversity, this effort is referred to as "mass bioprospecting".

3. Mass bioprospecting: contemporary models

The effort of the United States National Cancer Institute (NCI) in searching for anticancer agents from the biodiversity, in the present context, of plants, is one example of mass bioprospecting. In this effort, field explorations are largely guided by the so-called biodiversity or "random" collection approach, with ethnobotanical or ethnopharmacological information playing a minimal or no role. NCI launched its effort in 1955, and for the period of 1960–1982, about 114,000 extracts from an estimated 35,000 plant samples (representing 12,000–13,000 species) collected mostly from temperate regions of the world had been screened against a number of tumor systems (Cragg and Boyd, 1996; Cragg et al., 1996). A wide variety of compound classes were isolated and characterized. Clinically significant cancer chemotherapeutic agents that emerged from this program included paclitaxel (*Taxus brevifolia* Nutt. and other *Taxus* species, Taxaceae), hyocamptamine (topotecan), CPT-11 and 9-aminocamptothecin. The latter three compounds are semisynthetic derivatives of camptothecin (*Camptotheca acuminata* Decne., Nysseaceae) (Cragg et al., 1993). The program was extended from 1986 to 2004, with an emphasis on global plant collections and screening against tumor cell cultures. Although no provision was made in the recognition of intellectual property and and in the arrangement of benefit-sharing during the first phase of the NCT's effort (1955–1980), a "Letter of Intent," later evolving into a "Letter of Collection" (LOC), then a Memorandum of Agreement (MOA), was in place as an umbrella for the field operation of the NCI contractors in the second phase of NCI-sponsored explorations (1986–2004). The LOE/LOC/MOA contains provisions to recognize the ownership of genetic resources and an arrangement to share the benefits of discovery (Mays and Mazan, 1996; Hallock and Cragg, 2003).

In 1983, the NCT's mass bioprospecting effort was extended through the establishment of a National Cooperative Drug Discovery Group (NCDDG) program by the Developmental Therapeutics Program (DTP), Division of Cancer Treatment and Diagnosis (DCT). This program supports broad, innovative and multi-disciplinary approaches to the discovery of new, synthetic or natural-source-derived anticancer agents (Hallock and Cragg, 2003; NCI-DTP-DCTGCOB, 2005). In the programs that target biodiversity as the source of discovery, biodiversity-based collection approach also formed the basis of organism selection. In the NCDDG program, issues on IP and benefit-sharing were dealt with individually by each group. Recently, the overall effort and accomplishments of the NCDDG projects were reviewed (Hallock and Cragg, 2003).

Commencing in 1993, the International Cooperative Biodiversity Groups (ICBG), a program administered by the Fogarty International Center (IFIC), National Institutes of Health (NIH) and supported through funds from NIH, National Science Foundation (NSF) and US Department of Agriculture (USDA) Foreign Agricultural Service (FAS), started operation in an effort to integrate the goals of improvement of human health through drug discovery, incentives for conservation of biodiversity and development of new models of sustainable economic activity that focus on the environment, health, equity and democracy. The implementation of this program is based on the belief that the discovery and development of pharmaceutical and other useful agents from the world’s biodiversity can, under appropriate circumstances, promote scientific capacity development and economic incentives to conserve the biological resources from which these products are derived (Fogarty International Center, 2004). This unique effort is undertaken in such a way that local communities and other source country organizations can derive direct benefits from the effort and, ultimately, from their diverse biological resources, so that benefit-sharing may provide clear incentives for preservation and sustainable use of the biodiversity (Rosenthal, 1996, 1997; Rosenthal et al., 1999, 2002a,b, 2004a,b). The ICBGs’ drug discovery effort is targeted to a broad range of organisms, including five of the six recognized kingdoms of biodiversity: Eubacteria, Protosticta, Plantae, Fungi and Animalia (NIH News, 2003).

4. “Studies on biodiversity of Vietnam and Laos”: an ICBG program based at the University of Illinois at Chicago

A complete review of the ICBGs has been published (Rosenthal and Pezzuto, 1999). The University of Illinois at Chicago (UIC)-based ICBG, known simply as “UIC ICBG” or “Studies on Biodiversity of Vietnam and Laos” (Soejarto et al., 1999, 2002a,b, 2004a,b), serves as a model for the implementation of the ICBG principles, which are, ultimately, the principles of the United Nations Convention on Biological Diversity. In its current Phase II operation (2003–2008), this ICBG consortium consists of two US-based academic institutions (UIC and Purdue University), two Vietnamese research institutions (Vietnamese Academy of Science and Technology; and Cuc Phuong National Park), one Lao research institution (Traditional Medicine Research Center) and an industrial partner (Bristol-Myers Squibb, BMS). Although the drug discovery and development objective of the UIC-ICBG is to uncover biologically active molecules from plants of Vietnam and Laos as chemotherapeutic candidates for malaria, tuberculosis, AIDS, cancer and CNS-related diseases, the bioprospecting goals are more all-encompassing, namely: (a) to discover and develop new drugs from plants of Vietnam and Laos (as just stated); (b) to promote economic development among communities in the ICBG study areas...
and to promote institutional-infrastructure strengthening and human-resource development of host-country institutions; (c) to undertake biotic survey and to promote conservation initiatives in Vietnam and Laos. In other words, the UIC ICBG is a bioprospecting endeavor that is truly multi-disciplinary and multi-national, and truly integrates drug discovery, biodiversity conservation and economic development. Since space only allows a rather narrow discussion, addressing, specifically, the drug discovery and development activities, readers are directed to papers already published that document activities in response to the other two goals of the UIC ICBG’s bioprospecting effort (Hiep and Loc, 2000; Riley, 2001; Soejarto et al., 2004; ICBG/AP4, 2005).

Bioprospecting: activities

In the selection of plants consist of biodiversity-based collection (“random” collection) centered in the Cuc Phuong National Park in Vietnam, and ethnobotany-driven interviews on the medicinal uses of plants in Laos. Ethnobotanical interviews were also performed in Vietnam during the first Phase of the UIC ICBG project (1998–2003), among communities (Muong ethnic minority) in villages surrounding CPNP. Seven years after the start of the UIC ICBG project (1998–2005), 3331 plant samples have been collected from Vietnam (Cuc Phuong National Park/CPNP) based on biodiversity (“random”) approach, comprising >950 species of flowering plants identified to species level. Since the results of the taxonomic inventory of plants in this park indicate that 1926 species of Angiosperms (Soejarto et al., 2004c) are found at CPNP, >900 species still remain to be collected and screened. In addition, 960 plant samples (about >700 species) have been collected based on ethnobotanical/ethnopharmacological field interviews with Muong communities surrounding CPNP in Vietnam, and with ethnic communities throughout Laos. For every plant sample collected, a set of seven voucher herbarium specimens was collected for deposit at the consortium’s herbaria and at other herbaria of collaborating botanical institutions.

The drug discovery process in the UIC ICBG follows classic pharmacognosy methods, through sample extraction, high throughput screening of extracts in the target disease system, recollection of samples (same plant parts) of active species and bioassay-guided fractionation and isolation. CH2Cl2-soluble extracts or CHCl3- or CH2Cl2-soluble fractions from MeOH/EtOH extracts are assayed, after elimination of tannins and polyphenols, which are known to interfere with certain enzyme-based assays.

Extracts that have shown confirmed activity are fractionated/isolated using an effective combination of expertise and advanced separation technology protocol, typically based on the combination of flash (Isco CombiFlash SG100 Separation System), semi-preparative and preparative HPLC chromatography, while droplet counter-current systems are employed to maximize efficiency. Traditional methods include vacuum, gravity, flash or low-medium pressure column chromatography using a variety of adsorbents and media. Early LC/MS/MS-based dereplication strategies that favor novel bioactive molecules over non-specific, known compounds from mixtures are employed to support the bioassay-directed fractionation and isolation process. Chemical profiling by HPLC–MS is used, especially, for chemotaxonomically related species under investigation.

State-of-the-art physical and spectroscopic methods, such as high field nuclear magnetic resonance spectrometry (NMR), time-of-flight mass spectrometry (TOF-MS), chemical ionization (CI), field desorption and fast-atom bombardment (FAB) MS, are employed in structure elucidation of active compounds. Stereochemistry is determined by the use of ORD and CD, coupled with high-field 1H and 13C NMR spectroscopy, with appropriate two-dimensional (2D) and decoupling experiments. The use of single crystal X-ray crystallographic analysis is routinely performed to solve difficult stereochemistry assignments.

At the end, new compounds that are routinely isolated based on a particular biological activity are included in the UIC ICBG natural products library and incorporated in the NAPIS Database, the computerized documentation system utilized in the ICBG program, for periodic submission to the ICBG’s Global Data Center (GDC) based in Friday Harbor, Washington State. These compounds may eventually be exposed to multiple screens across the ICBGs.

5. UIC ICBG bioprospecting effort: results

Of the 3331 samples collected, extracts of 2309 (comprising approximately 800 species) have been screened in anti-HIV, antimalaria and anti-TB disease systems, as well as in a tumor cell lines panel. Twenty-two recollections of active samples have been made and approximately 280 pure natural products of varying degrees of structural complexity and/or biological activity have been isolated. Aside from the discovery of biologically active compounds, the UIC ICBG studies have also made significant contribution to the knowledge of natural products chemistry through the isolation and elucidation of 80 new secondary metabolites and novel chemical entities, reported for the first time from higher plants. This high number may have been the result of rigorous prioritization criteria in the project, based on pre-existing biological and chemical information (both at species and generic levels), as well as on a chemical dereplication strategy using the resources and expertise available at UIC. The chemical diversity of these new natural products includes alkaloids/amides, macrocyclics, lignans, neolignans, butenolides, phenylpropanoids, terpenes, norterpenes, triterpenes and steroids (Zhuang et al., 2001, 2002a,b; Chien et al., 2004).

Ten of the 80 new chemical entities have a novel carbon skeleton, being described and communicated for the first time, all of which are structurally related anti-HIV sesquiterpenes (litsea verticillata) isolated from Litsea verticillata.
medicinally (Penso, 1976; Schippmann et al., 2002), of which between 20,000 and 55,000 species of plants have been used of biodiversity (15%). Estimates have been made that (Hammond, 1995), plants represent the second largest source of global biodiversity, respectively. It is reasonable to assume the fungi and the protists [protoctists] represent 4% and 5%, while animalia, excluding the arthropods, comprise 13%, while the remaining 62% of the earth’s biodiversity. Despite the fact that the number of insect species is estimated to be between 2,000,000 and 100,000,000 (Hammond, 1995), this group remains relatively untouched as a source of novel compounds by the drug discovery community. Animal species [Kingdom Animalia], comprising about 1,085,000 species, including insects, make up the largest portion (75.4%) of the earth’s biodiversity (Hammond, 1995).

Examination of the statistics on number of described species shows that arthropods (Kingdom Animalia), comprising about 1,085,000 species, including insects, make up the largest portion (75.4%) of the earth’s biodiversity (Hammond, 1995). Insects alone, estimated at 950,000 species, comprise 62% of global biodiversity. Despite the fact that the number of insect species is estimated to be between 2,000,000 and 100,000,000 (Hammond, 1995), this group remains relatively untouched as a source of novel compounds by the drug discovery community. Animal species [Kingdom Animalia], excluding the arthropods, comprise 13%, while the fungi and the protists [protoctists] represent 4% and 5% of global biodiversity, respectively. It is reasonable to assume that the overwhelming majority of fungal species have yet to be isolated and tested for the production of biologically active compounds, even though members of the order Actinomycetales, like Streptomycetes, have been the most prominently known microbial producers of natural products.

Based on the number of known species (300,000–500,000) (Hammond, 1995), plants represent the second largest source of biodiversity (15%). Estimates have been made that between 20,000 and 55,000 species of plants have been used medicinally (Penso, 1976; Schippmann et al., 2002), of which only a small portion has been investigated for drug purposes. Among those that have been investigated are plant species that produce important drugs such as quinine, reserpine, tubocurarine, vincristine, vinblastine, pilocarpine, atropine, morphine, cocaine, to mention a few. Overall, only 15–20% of terrestrial plants have been evaluated for pharmaceutical potential. Consequently, plants, including uninvestigated medicinal plants, continue to represent a significant pool of raw material for the discovery of new drugs.

With the biodiversity statistics presented above, the world’s biodiversity and the ethnomedical and ethnopharmacological treasure house that remains in store will continue to present an attractive target for future mass bioprospecting effort.

### 6. Mass bioprospecting in the future

Estimates place the number of species of organisms at between 1,392,485 (Wilson, 1988) and 1,750,000 (Hammond, 1995), classified into Kingdoms Eubacteria (bacteria, cyanobacteria “blue-green algae”), Archaea (halobacteria, methanogens, coccites), Protocista (protozoa, “algae”), Plantae (land plants), Fungi (molds, lichen-forming, yeasts, mushrooms) and Animalia (mesozoa, invertebrates, mammals) (Hammond, 1995).

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Central in any mass bioprospecting is the drafting and signing of an international agreement or Memorandum of Understanding (MOU) or MOA, that should cover issues on access to the genetic resources [the biodiversity], on IP related to discovery, on the sharing of the benefits as part of the process (short-term) and in the event of discovery and commercialization of a product (long-term), as well as on the conservation of the biological resources for the future generations. When ethnomedical or ethnomedical approach is utilized, additional specific requirements that relate to prior informed consent (PIC), recognition of Indigenous Intellectual Property (IIP) and Indigenous Intellectual Property Rights (IIPR), as well as short- and long-term benefit-sharing are “priority” items to take into account.

Collection methodology that facilitates recollection, in the event of active species, is essential in order to ensure a reliable supply of biomass for larger scale compound isolation to meet the requirements of in vivo and late-stage preclinical studies. This includes good field documentation, use of Global Positioning System (GPS) to pinpoint site locations, mapping of sites and the ready availability of superior computer database support. As an example, the field data for the UIC ICBG program are posted on the Internet in the “Atlas of Seed Plants of Cuc Phuong National Park” (http://uicichbg.pharm.uic.edu). This information is also made available in hardcopy form (Soejarto et al., 2004c). Good collection
methodology is especially important for marine sites, though collection technology is different (Wright et al., 1996). Needless to say, precise taxonomic identification of organisms involved either in the field, or with subsequent support from taxonomic specialists, is crucial for accurate recollection of organisms or targeted collection of specific organisms (Soejarto, 1996).

Another important tool is plant tissue or cell culture technologies, which are suited for scale-up production of bioactive metabolites once they have been determined to be of interest (DiCosmo and Misawa, 1995; Kirakosyan et al., 2004). In the UIC ICBG, plant cell culture has been applied to eliminate the uncertainty of re-accessing native plant samples that have exhibited interesting chemistry. Further development of sustainable supplies of compounds for clinical trials or commercialized drugs is critical, and may proceed by exploring sustainable harvest methods, cultivation (including aquaculture), microbial fermentation, genetic engineering and semi-synthesis or synthesis of candidate drugs or analogs (Wender et al., 1999). Such effort will ensure adequate supply of the compound while protecting the source organism and its habitat from overexploitation. Planning for sustainability should begin early in product development (Cragg et al., 1993; Cragg, 1998). In the UIC-based Vietnam-Laos ICBG, plant tissue culture is also being used to produce biomass of rare and threatened species at Cuc Phuong National Park, for purposes of both biological evaluation and increasing species population in the park. It is also intended to promote economic development among communities in the ICBG research site by helping members of the community to generate income from distributed starter plants, in cases of economically valuable species.

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