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21st Century Natural Product Research and Drug Development and Traditional Medicines

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Abstract

Natural products and related structures are essential sources of new pharmaceuticals, because of the immense variety of functionally relevant secondary metabolites of microbial and plant species. Furthermore, the development of powerful analytical tools based upon genomics, proteomics, metabolomics, bioinformatics and other 21st century technologies are greatly expediting identification and characterization of these natural products. Here we discuss the synergistic and reciprocal benefits of linking these ‘omics technologies with robust ethnobotanical and ethnomedical studies of traditional medicines, to provide critically needed improved medicines and treatments that are inexpensive, accessible, safe and reliable. However, careless application of modern technologies can challenge traditional knowledge and biodiversity that are the foundation of traditional medicines. To address such challenges while fulfilling the need for improved (and new) medicines - we encourage development of Regional Centres of ‘omics Technologies functionally linked with Regional Centres of Genetic Resources, especially in regions of the world where use of traditional medicines is prevalent and essential for health.

Introduction and Background

Despite the great synthetic diversity derived from the development of combinatorial chemistries and high-throughput screening methods over the past fifty years, natural products and related structures continue to be extremely important elements of pharmacopoeias. Looking forward, natural products and related structures are likely to become even more important for development of improved and new medicines, due to the variety of functionally relevant secondary metabolites of microbial and plant species whose chemical and genetic diversity are being revealed by ultra fast DNA sequencing and related genomics and bioinformatics tools [1-4].

Heretofore, methods for identifying and characterizing the activities of secondary metabolites have been inefficient and often tedious, but recent advances in genomics, informatics, and associated 21st century ‘omics technologies are dramatically accelerating the pace of discovery and analysis. Sophisticated fractionation methods hyphenated to modern spectrometries and spectroscopies as described in this issue (cite reviews by Reynolds[5], Hamburger[6], Bucar[7], and Wurtele[8]) can define the metabolomes of cells, tissues and even organisms. Multivariate analyses[9] and network modeling [10-12] enable comprehensive identification and evaluation of natural product diversity and functionality; and when integrated with systems approaches, it is possible to profile molecular changes caused by mutation and by pathogens and other environmental stressors, and thus to predict the targets and mode(s) of action and toxicities of natural products and derivatives [13, 14].

Considerable synergy and benefit for the development of improved medicines and new drugs can come from linking these powerful scientific tools to robust ethnomedical and ethnobotanical studies of traditional medicines. However, ethical and socio-economic challenges also must be addressed for the development of improved medicines and new drugs to be achieved while also benefiting those that currently rely upon traditional medicines and accompanying natural products for health and well being.

As background, we rely upon these recent contributions: Corson and Crews [15] summarize classical approaches for identifying and studying single active agents of traditional medicines - but these methods are clearly very limited when viewed in context of 21st century 'omics technologies. Ulrich-Merzenich, et al. [16] and Heinrich [17] describe how genomics, proteomics, metabolomics and systems biologies are contributing to ethnopharmacy, and Prasain and Barnes [18] focus upon the contributions of 'omics technologies to understanding and validating traditional medicines. These and other similar reviews document the value of 'omics technologies for advancing analysis of natural products (especially from plants), but in our view the *reciprocal and synergistic* benefits of linking robust ethnomedical and ethnobotanical studies of traditional medicines with 21st century 'omics technologies have not been adequately considered.

Here, we focus upon development of improved and new medicines and treatments, especially for ancient diseases such as tuberculosis (Tb) and malaria that continue to plague mankind, and for which traditional medicines have been and will continue to be used.

Tuberculosis and malaria

Throughout recorded history, *Mycobacterium tuberculosis* has been a leading cause of human morbidity and mortality. With the biomedical advances of the twentieth century, global Tb incidence declined, but the disease has resurfaced with even more vigour in immune-compromised peoples living with HIV/AIDS, and increasingly with diabetes. Presently, about two billion persons are infected or are at risk of *M. tuberculosis* infection, which is currently responsible for 2.4% of worldwide mortality[1920]. Malaria, another ancient and important infectious disease is caused by various *Plasmodium* species and ranks 5th among causes of death. At particularly high-risk for malaria are children, pregnant women and the many who are immune-compromised; in highly malaria-endemic areas, a child dies every minute from malaria[21]. In addition to the immediate impact on individuals and families, these ancient diseases have huge economic and social costs that threaten civil societies [22].

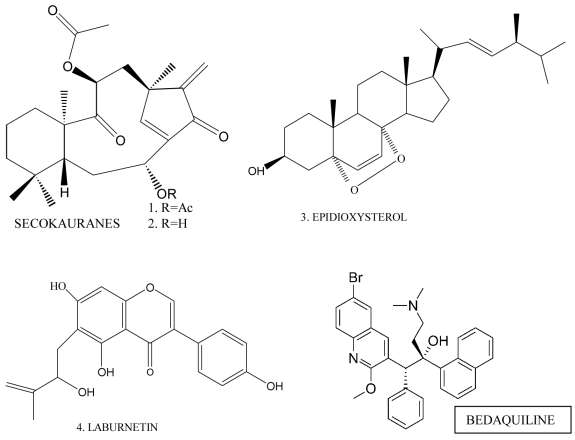
Current therapies for Tb require long regimens with significant side effects and toxicities. Consequently, treatments suffers from poor adherence and are compromised by the emergence of multidrug and extreme drug resistant *M. tuberculosis* [23]. For malaria, artemisinin combination therapies (ACT) have reduced disease prevalence and mortality, but the requisite medicines are not accessible to many that need them and their dosing is complex, so monotherapies are still widely used that promote genetic resistance[21, 24]. New drug scaffolds and treatments that address these shortcomings are critically needed for both Tb and malaria (and other extant and emerging diseases). Importantly, the new treatments should have multiple independent targets and must be accessible and affordable [25, 26]. These are often properties of traditional medicines, so better understanding and use of traditional medicines may significantly benefit health and well being.

Significant challenges in developing new treatments for Tb and malaria arise from the complex biology of the pathogens, the expense of research, the lack of financial incentives and inadequate delivery systems [27-29]. A "systems biology" approach that integrates epidemiology with 'omics technologies has been suggested to strengthen such efforts [30]. We

recommend inclusion in such ‘systems’ approaches, additional elements comprisin robust ethnobotanical and ethnomedical analyses of traditional medicines. Such a ‘hat trick’ of ‘systems studies’ could expand upon the well-established paradigms of important single agent drugs such as aspirin, codeine, quinine and artemisinin[31], by adding multicomponent mixtures of treatments derived from traditional medicines that also can be accessible and affordable to those in resource poor regions.

After many years of research, some promising candidates for anti-mycobacterial drugs recently have reached clinical trials [32, 33] and additional candidates will derive from the elucidation of the *M. tuberculosis* genome [34] and its transcriptome and metabolome and structural and system biology modeling [35, 36] through *in silico* analyses [37, 38] and from insights into the infection and survival pathways adopted by *M. tuberculosis* [39-41], as also of host-microbe interactions [42], and informatics resources [43,44-46]. Very recently, the U.S Food and Drug Administration (FDA) approved the first new Tb drug in more than 40 years [47]. Bedaquilin (shown below) was discovered in 2005[48] and was assessed by clinical trials of multidrug-resistant Tb patients[49]. However, treatment requires drug-sensitivity tests which are costly and time-consuming and therefore may not reach many that suffer from Tb in low-income countries.

Numerous natural products in traditional medicines inhibit *M. tuberculosis* [50] (salient examples are the secokaurenoids [51], epidioxysterol [52], and the isoflavonoid laburnetin [53] shown below), but we are unaware of any current efforts to use these compounds as scaffolds for new drugs. Nor are we aware of concerted efforts to screen other antimicrobial compounds [53] that could become new anti-mycobacterial drug scaffolds.



Similar research advances are being made in the analysis of factors contributing to malaria, including the elucidation of the genomes of *Plasmodium* species [54-57] , analysis of parasite-host interactions [58], identification of vaccine targets [59-61], transcriptomic, metabolomic and proteomic analyses [62, 63], and new insights into the stages of parasite development [64-66]. Moreover, the mechanisms of artemisinin action against *Plasmodium* spp. are being described and correlated with regions of the *Plasmodium* genome [67-69] . Genome-scale proteomics and structural biology are helping to elucidate protein structures of potential targets which should facilitate the development of vaccines and new drugs [70]. Approaches to further understand the dynamics of artemisinin action and its targets will likely be developed, as has occurred with drugs targeting influenza, bacteria, HIV, and cancer[71-74, 75] and should provide deeper insights into the mechanisms for pathogen resistance.

Speeding development of 21st century medicines and treatments

The development of artemisinin and related antimalarial compounds serves as a modern paradigm for the value of traditional medicines in drug discovery, and we assert the potential exists for additional discoveries of similar importance: of the estimated 250,000 – 500,000 extant plant species, only a fraction have been scientifically investigated for biological activity[76-79]. Unexplored are untold numbers of species that are likely to be included in traditional medicines [80]. Plants from widely separated regions of the world that are components of traditional medicines used to treat specific conditions such as malaria are phylogenetically clustered [81]; this principle has been recently described for *Pterocarpus,* which has significant cross-culture patterns that can inform drug development and supports the value of linking robust ethnobotanical and ethnomedical studies with 21st century 'omics technologies and systems analyses [82-84] to speed identification of functionally relevant bioactivities .

Inclusion of traditional medicines in development of 21st century treatment paradigms can help assure their convenience, acceptability and accessibility [85] . Furthermore, pharmacological synergism, a principle employed by many traditional medicines lessens the likelihood of development of genetic resistance by the pathogen or disease against drug monotherapies. Synergy research inspired by a "reverse pharmacological approach" [86], could lead to a "new generation of phytopharmaceuticals" [87]. The use of powerful 'omics technologies facilitates disentangling such complexity[88, 89]: metabolomics analyses enable profiling of major and minor metabolites and bioactive components that contribute to synergism; and computational approaches for analysis of multiple-activity networks have become powerful tools for defining the principal components of mixtures with synergistic modes of action, for prediction of drug metabolism and toxicity, and for high-throughput prioritizing of agent combinations [90]. Data mining approaches to identify active compounds in mixtures of natural products are being developed [91] and will be essential for the development of effective multiple-agent drugs from traditional medicines.

While U.S. requirements for regulatory approval for health claims made for multicomponent medicines present significant challenges for the development of effective multiple-agent drugs from traditional medicines, this is less so for Europe, and especially not for regions of the world highly impacted by Tb and malaria (and in which there are strong traditions of traditional medicine use). As described below, with the establishment of regional research facilities to confirm the safety and efficacy of traditional medicines through the use of 'omics tools and robust ethnobotanical and ethnomedical data, significant improvements in development of improved medicines that are accessible and affordable can be expected.

A significant challenge in anti-Tb drug discovery and development is the lack of suitable animal models that can help predict clinical outcomes of infection[25]. Traditional medicines that have been used for generations can be said to have undergone preliminary preclinical and clinical assessment and thus, with appropriate observational data for safety and efficacy, should offer leads that can supplement or even bypass testing in animal models [31, 92]. In turn, application of 'omics technologies and systems biologies can provide independent evidence for safety and efficacy of traditional medicines [89, 93, 94].

Use of 'omics technologies for medicinal plant improvement

21st Century 'omics technologies also can advance the synthesis and production of natural products. An excellent example is artemisinin, traditionally derived from *Artemisia annua* [95, 96] and very recently, due to the application of genetic technologies, from microbial synthesis [97-100]. Traditional medicines containing *A. annua* have been used for thousands of years and as a consequence, the plant is important in healthcare, agriculture and

commerce [101-103]. However, artemisinin yields from most outbred varieties is very low, so 'omics technologies also are being employed to increase yield [96]: the synthesis and regulation of artemisinin metabolic pathways have been described [104, 105], and significant attempts have been made to clone biosynthetic cDNAs and ESTs [106], characterize transcription [107], identify key genes [108, 109], and profile expression and metabolite levels [110, 111112113].

Plant natural products are intertwined in societies' cultures, healthcare systems, and economies [114, 115], whose indigenous knowledge [116] can inform natural product research and further development [117, 118]. Attention to the conservation of these cultural and biological resources has been assumed by governments and NGOs [119-121] as the livelihoods of people who rely on medicinal plants, especially farmers, marketers and traditional healers, are dependent upon improvement of natural products and related resources [114]. Switching to an improved variety of *A. annua* potentially benefits growers, provided that market demand and prices are assured [122, 123]. The cost of artemisinin production in developing countries is usually low, creating competitive markets for the products; for example, in Vietnam, the cultivation of *A. annua* produced approximately 13 tons of artemisinin at $1000-2000 per ton [124]. Other plants with promising medicinal properties also can become potential sources of income[125, 126].

Technological development affects the livelihoods of people who participate in the analysis, production and distribution of natural products [114, 118, 127] and can be positive or negative [128, 129]. For example, the significant advances in synthetic and microbial artemisinin manufacturing and in *A. annua* crop yield [113, 130] can contribute to increasing artemisinin supply [131] and may result in decreased cost of artemisinin production for artemisin combination treatments (ACT) [132] . However, while significant improvement in malaria treatment in the early 1990s was associated with increased production of artemisinin in Vietnam [133, 134], artemisinin supply from *A. annua* cultivation is predicted to meet ACT demand in 2012, hence further increases in artemisinin production are not likely to provide equivalent improvements in malaria treatment [128, 129, 135].

Consequently, key recommendations from the RBM/UNITAID/WHO have been to improve communication and flexibility in the supply chain for artemisinin, rather than to increase yield and production [135]. If microbial production of artemisinin becomes significant, plant-derived artemisinin will have to compete with the microbial product, leading to a significant impact on the livelihoods of people who rely on the crop for income.

Preservation of Traditional Knowledge, Biodiversity, and Access and Benefit Sharing of Genetic Resources

The Convention for Biological Diversity (CBD) articulates principles intended to protect traditional knowledge and biodiversity and to facilitate access to genetic resources and the fair and equitable sharing of benefits arising from their utilization. However, these principles have not been translated into reality. Disappointingly, many of the Access and Benefit Sharing (ABS) regimes that developed from the CBD are proving to be significant barriers to the development and utilization of genetic resources, and also poorly define the traditional knowledge systems and the owners that might benefit from ABS regimes[136-138].

Recommendations regarding responsibilities and procedures for implementation of the CBD principles and for assuring compliance are articulated by the Nagoya Protocol, which clarifies the scope of the CBD to include research and development of gene sequences and natural products and the traditional knowledge associated with these genetic resources [139]. However, much remains to fulfill the vision of the CBD. Parties must develop implementing rules for ratification of the Nagoya Protocol, and there is need for constructive dialogue

between parties that view themselves as the 'providers' or 'users' of genetic resources and traditional knowledge. Such dialogues will be stimulated by development of cooperative research agreements and exchanges of best practices [139] and concomitant commercial successes guided by the CBD principles.

The 'fair and equitable sharing of benefits' principles articulated by the CBD are not solely concerned with the sharing of results, products of commercialization and other outcomes of the use of Genetic Resources, but also are meant to include participation in research and development and transfer of technologies [138, 140]. Consequently, those who rely primarily upon traditional medicines for their healthcare and livelihoods also should directly benefit from their more detailed analysis and development[141]. Returning to some of the literature cited earlier, Ulrich-Merzenich, et al.[16] recommend use of 'omics technologies for development of local resources that improve primary care; and similarly, Prasain and Barnes [18] suggest the development of *integrative* global healthcare systems combining traditional and modern medicines. This latter goal has been espoused by the World Health Organization but not without controversy. Hollenberg, et al. [142] suggest that traditional medical practices can fruitfully coexist with public health and other medical science innovations, but also caution such relationships are especially vulnerable. Parties should appropriately gauge the outcomes of development and encourage only those applications that can be realized without harmful consequences.

Regional Scientific Research and Development Centres

Parties that wish to apply 'omics technologies to natural product research and drug development are faced with significant start-up and ongoing costs for infrastructure and for trained personnel. These costs can exceed tens of millions of dollars for space and for equipment, and millions of dollars yearly for maintenance of the space and equipment and for training staff. Costs for 'omics technologies experimentation can exceed tens of thousands of dollars per protocol. While many 'omics-related informatics resources are readily accessible, the technology and computing infrastructure and properly trained staff required to ensure that the informatics resources reach the potential users can be costly. Many low- and middle-income countries are unlikely to be able to provide such resources without external investments [143, 144]. However, those parties that lead the development of public-private partnerships and achieve initial successes are likely to secure long-lasting temporal and market advantages.

To benefit from 'omics technologies, countries must develop scientific training programs that strengthen know how. This is occurring in Viet Nam, where the Vietnam Academy of Science and Technology (VAST) has built strong networks in research and education with 28 international institutions of 16 countries [145], and the Oxford University Clinical Research Unit in Viet Nam (OUCRU) is providing opportunities for scientists to conduct research programs focusing on malaria, tuberculosis, HIV/AIDS and other major diseases [146]. Together with such efforts, building research capacity to study traditional knowledge and traditional medicines is vital.

Analogously, the network of Botanic Gardens in South East Asia, established in 2004 by the Botanic Gardens Conservation International (BGCI) emphasizes conservation of biological diversity and related research and education[147]. BGCI has built similar strong networks to secure biodiversity, enable training and influence decision making and policy regarding CBD[148]. We suggest that these centres might link to regional research centres with 'omics capacities to expedite natural product discovery and drug development. Arguments in favour of regional centres as means of drug development and commercialization have been made by others [138, 149], but we expand upon these suggestions by combining development of

‘omics technologies and biodiversity and indigenous knowledge conservation, access benefit and sharing of resources.

For Africa the BecA-ILRI Hub located in Nairobi, Kenya and managed by ILRI as one of four biosciences centres of excellence that are part of the African Union-New Partnership for Africa’s Development (AU-NEPAD) African Biosciences Initiative is developing ‘omics infrastructure. BecA-ILRI has been created under the Comprehensive African Agricultural Productivity Programme (CAADP) to service the needs of countries in eastern and central Africa. Analogous investment and partnerships in Ghana could provide similar resources to western Africa[150]. In southern Africa, outstanding ‘omics infrastructure occurs in South Africa’s major research universities and government research agencies, and draft government policies on African Traditional Medicine provide a framework for the registration and regulation of genetic resources, indigenous knowledge and African traditional medicines, and their study through ‘omics technologies. Rigorous clinical evaluation of the more widely used traditional medicines of southern Africa are occurring through partnerships between traditional medical practitioners and biomedical and social scientists[151]. These partnerships create environments that can guide the choice of traditional medicines to be studied by ‘omics techniques.

Altogether, these efforts should greatly improve treatment programs for HIV/AIDS, Tb, malaria and the chronic diseases for which traditional medicines have been employed for generations, and have much potential to contribute solutions.

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