BASIC NEGLECTED RESEARCH AGAINST AMR: WHAT IF PLANTS PROVIDED A SOLUTION?

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With the continued spread of antimicrobial resistance, new anti-infectives are needed with novel mechanisms of action and the potential to slow or circumvent resistance. The vast and diverse library of chemicals contained in plants, termed phytochemicals, represents a promising and largely untapped source of anti-infectives. This article discusses the highly favourable attributes of plants and their chemicals for drug discovery as well as their advantages over other natural products. In decades past, phytochemicals have not been comprehensively utilized in drug discovery efforts due to a combination of both real and perceived challenges. These challenges and their solutions are also discussed along with new technologies and recent discoveries that have revealed some of plants' tremendous potential as sources of anti-infectives. In recent years, attention has begun to increasingly fall on plants and phytochemicals for drug discovery efforts. It is clear that a re-emergence of phytochemicals in anti-infective drug discovery is inevitable and holds great promise for the development of new therapies against antimicrobial resistance.

Throughout history, plants have been the main resource for medicine for peoples across the world. Even today, it is estimated that in some regions, 80% of the population relies on traditional medicine for their medical needs *(1)*. A decoction of certain fruits may be drunk to relieve stomach ulcers, or a cataplasm of certain leaves may be used to dress infected skin to cure the infection. Countless specific examples of such treatment strategies exist in the traditional medical practices of communities across the world. For over a century, due to improvements in our understanding of biology and chemistry and in the instrumentation to study the two, evidence-based medicine has revolutionized the way in which diseases are treated. Indeed, disease pathology and drug mechanisms of action can be deduced to the molecular level, opening many doors to the discovery of new medicines. Now, instead of medicating a patient with a preparation of fruits or leaves which have been observed to work somehow, we can medicate with a pill or injection of an isolated chemical experimentally known to exert certain biochemical changes in the body. While there are many obstacles scientists face in developing new drugs, a foundational problem is the selection of sets or libraries of chemicals to explore in the effort to find chemical hits that favourably perturb a certain pathological biochemical process. This where plants and the chemicals they produce become of immense value.

Plants as a source of chemical diversity

Plants are sessile, and as such are incapable of movement to protect themselves from predators. Unlike animals, a plant cannot bark to scare a predator, scratch to defend itself, or run away to protect its life. Instead, for the purpose of self-protection and communication with other organisms in the environment, plants produce a large array of diverse chemicals called secondary metabolites. Some plant secondary metabolites are used for attracting pollinators, while others are used for repelling insects, killing infecting fungi, and countless other functions. As such, any single plant in fact represents a library of hundreds to thousands of architecturally and stereochemically complex chemicals, termed phytochemicals *(2)*. What is more, many of these phytochemicals intrinsically act as anti-infectives: the fact that a cataplasm of certain leaves is capable of curing a skin infection indicates that anti-infective chemicals are indeed produced by and present in those leaves. As it turns out, evidence-based medicine has still barely scratched the surface of this botanical chemical space, even with the many clinical successes of phytochemical-based drugs *(3)*.

Natural products comprise all chemicals produced by living

organisms, and phytochemicals fall under this category. These two terms are typically used to refer not to primary metabolites (amino acids, sugars, and other chemicals directly responsible for life) but to secondary metabolites (produced as an adaptation to the ecosystem). Natural products share several important characteristics which make them extremely important for consideration in drug discovery efforts. Natural products inherently fall into regions of the biologically relevant chemical space, which refers to all chemicals that are biologically active *(4-7)*. This in fact makes sense, since the secondary metabolites produced by plants and other organisms have evolved in the context of surrounding organisms on which they act. Natural products exhibit a high tendency to be metabolite-like, and so they are largely compatible with cellular transport systems to gain entry into tissue *(8)*. On top of this, natural products possess massive chemical and structural diversity unmatched by synthetic small molecules, providing endless possibilities for drug scaffolds and pharmacophores *(9)*. For example, it has been demonstrated that 83% of natural product core ring scaffolds were not present in commercially available screening libraries and molecules *(10)*. Additionally, a retrospective analysis of one company's high throughput screening (HTS) campaigns showed that inclusion of natural products would have significantly improved hit rates *(11)*. And with all this, approximately 60% of the >126,000 compounds in *The Dictionary of Natural Products (12)* satisfied Lipinski's rule of five and are drug-like *(7)*. On the whole, natural products and derivatives thereof make up more than half of all the drugs in clinical use across the world, with at least one quarter of the total being contributions from plants *(13)*.

Advantages of phytochemicals over other natural products

While it is important to explore all types of natural products in drug discovery efforts, phytochemicals, or the chemicals plants produce, present key advantages. First and foremost is the advantage of traditional medicine, which enables a targeted, ethnobotanical drug discovery methodology (Figure 1). Traditional medicinal knowledge has played an important role in human history across the world and ethnobotanists have used this body of knowledge to identify plants and parts thereof with traditional medicinal uses against specific diseases. Because of this, a study that aims to discover hits against fungal infections, for example, could narrow the screening library down to extracts of or chemicals produced by plants that are documented to have been traditionally used against such infections. In this way, the drug discovery approach is targeted as opposed to being a random screening of plant species.

Another advantage is that plant extracts are relatively simple to make in large quantities, especially for those species that are abundant in the wild or amenable to cultivation. There are numerous ways to obtain an extract of a plant or plant part; examples include steeping plant material in an organic solvent such as methanol or ethanol or performing a decoction by boiling in water. More advanced methods include sonication assisted extraction, microwave extraction, and more *(14)*. Once the extracting solvent has been removed by evaporation and freeze-drying steps, what remains is the large portion of the plant part's chemical library that was soluble in the extracting solvent.

Plant extracts represent an exciting source of new chemical entities in drug screening due to the potential presence of multiple active chemicals and active chemicals that act synergistically. Indeed, vincristine and vinblastine are two alkaloids present in the Madagascar periwinkle, *Catharanthus roseus*, which exhibit potent anti-cancer activity and are approved by the United States Food and Drug Administration (US FDA). Additionally, some very intriguing results have been reported on the therapeutic use of the whole plant of sweet wormwood, *Artemisia annua*, the source of hugely successful anti-malarial, artemisinin. A study of a rodent model of malarial infection showed that oral delivery of the dried leaves of whole plant *A. annua* reduces parasitemia more effectively than a dose of pure artemisinin matching the whole plant content *(15)*. The administration of artemisinin in this whole plant form was documented to result in a 40-fold increase in the drug's bioavailability. From extracts of whole plants, single chemicals that contribute to bioactivity are discovered through the process of bioassay-guided fraction. In this framework, crude plant extracts are fractionated, with highly active fractions identified in bioassays subject to further iterations of fractionation until a highly enriched fraction or chemical that is active is isolated.

Overcoming challenges and new opportunities

Despite the past success and great promise of phytochemicals, they have experienced diminished representation in drug discovery efforts in the past three decades. As explained in numerous review papers *(9, 16-18)*, this lack of representation is not associated with poor promise but rather a combination of factors: embracing of combinatorial chemistry as sufficient to provide all the needed chemical diversity, perception that phytochemicals and natural products in general are incompatible with HTS, re-isolation of known chemicals, difficulty of performing chemical modifications on more complex structures, isolation of individual compounds from complex plant mixtures, and difficulty of acquiring foreign plants.

In fact, over the years these perceptions and difficulties

have been overcome. Combinatorial chemistry has provided disappointing output in practice *(19)*, and now high throughput screens of similar synthetic chemicals often suffer from low hit rates *(20)*. With this have come two assessments:

- For a screening library, the characteristic of diversity within the biologically relevant chemical space is of greater importance than library size *(8)*; and
- Biologically relevant chemical space is better covered by natural products than by synthetic compounds *(4-7)*.

In order to increase the hit rate of plant extracts in HTS, methods such as pre-fractionation have been used *(21-23)*. These methods aim to remove groups of chemicals such as very hydrophilic and hydrophobic chemicals from extracts before screening since they have a very low likelihood of being biologically active. In fact, from nine screens of a microbial natural product library comprised of 1,882 active cultures, 79.9% of the activities were observed in the fractions while only 12.5% was found in the crude culture extracts *(24)*. Also established are dereplication strategies to prevent re-discovery of known chemicals *(25-27)*. Such strategies make use of a combination of analytical methods including ultraviolet spectroscopy, tandem mass spectrometry (MS), and nuclear magnetic resonance (NMR) in order to ensure correct basic structure determination. In terms of medicinal chemistry, the field has advanced to where modifying complex structure is no longer as difficult *(18)*. Finally, acquiring foreign plants can be done through established international procedures and best practices. Through the United Nations Convention on Biological Diversity, a treaty signed in 1992 by more than 150 governments, and the Nagoya Protocol, nations hold sovereign rights over their traditional medicines and should receive equitable benefits in exchange for sharing them and for the successes that result from their utilization *(28)*.

The realm of anti-infective drug discovery, then, is set for a re-emergence of phytochemicals and natural products in general. Not only are old challenges falling, but new technologies have opened the doors to innovation. One of the aims of metabolomics is to analyze the total metabolites contained in an organism under specific conditions at a specific time. This technology is aided by instrumentation such as MS and NMR that allow for masses and structures of chemicals in a sample to be elucidated. Combining this with genomics approaches allows for the identification of genes and their contributions to metabolite production. Taken together, these two technologies can inform scientists who seek to genetically engineer plants with an optimized biosynthetic pathway for the production of a particular metabolite *(29-31)*. Indeed, the use of plant biotechnology to "improve or enhance the inherent economic or culturally valuable traits of plants as described and influenced by ethnobotany" falls under the domain of the newly expanding field of ethnophytotechnology *(32)*.

Metabolomic analysis of botanical fractions can aid in identifying fractions most likely to contain bioactive constituents *(33)*. Such analyses can be paired with rapidly-improving information technologies to utilize online databases, allowing for deduction of potential bioactivities based on unique features of compounds. Metabolomics has also allowed for the study of the complex chemical mixtures of various plants prescribed in combination under traditional Chinese medicine and their effects on complex biological systems *(34-36)*.

Phytochemicals as a promising source of anti-infectives

The above discussion speaks to the potential of phytochemicals for drug discovery in general. Upon this foundation, an understanding can be built concerning their potential not just for the discovery of novel anti-infectives but for the circumvention of antimicrobial resistance. Keeping all the above in mind, a perplexing statistic remains: While 69% of all US FDA-approved antibacterials are natural products or derivatives thereof, 97% of these come from microbes while only 3% come from plants *(37)*. In fact, this statistic reflects not the potential of phytochemicals for the development of novel anti-infectives but rather a combination of three phenomena:

- The actual aforementioned neglect of phytochemicals as drug sources due to perceived and real challenges;
- The tiny portion of total plants studied to date for anti-infective drug discovery; and
- The challenges faced by laboratories across the world, particularly academic, that have identified anti-infective activity in plant extracts and need to progress to chemical isolation and lead optimization.

Over time, not only can the latter two challenges be expected to resolve, but phytochemicals can be expected to regain the attention of scientists involved in anti-infective drug discovery. In general, consideration for phytochemicals is beginning to increase, with approximately 15% of the drug interventions in 2013 in the ClinicalTrials.gov database being plant-related, 60% of which coming from just 10 taxonomic families *(38)*.

Innovation is needed in order to slow down or circumvent the development of antimicrobial resistance, and this involves developing anti-infectives with different mechanisms of action capable of attenuating microbial pathogenicity. To this extent, a number of plant extracts have been identified. For example, an enriched extract of the Elmleaf blackberry, Rubus ulmifolius, demonstrated a potent ability to improve antibiotic efficacy against staphylococcal biofilms *(39, 40)* and

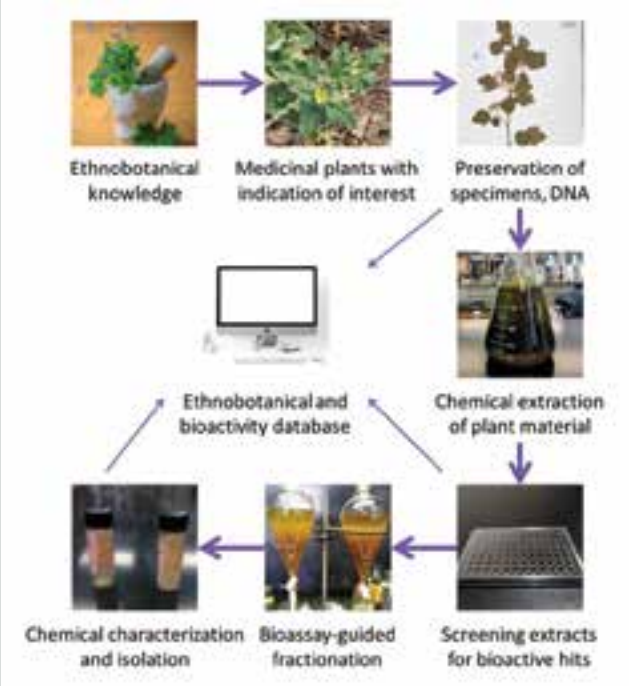
to eradicate pneumococcal *(41)* biofilms. Current research on this extract aims to develop it as a medical device coating and antibiotic adjuvant. Another example is an enriched extract of the European Chestnut, *Castanea sativa*. This extract demonstrated a potent ability to inhibit the quorum sensing system of cell-to-cell communication in methicillin-resistant *Staphylococcus aureus* (MRSA) *(42)*. This system of communication between cells is the chief modulator of pathogenicity in *S. aureus*; consequently, treatment with this *C. sativa* extract severely impaired MRSA pathogenesis in a mouse skin infection model without manifesting local or systemic toxicity *(43)*. Importantly, both of the plants mentioned here were included in preliminary screenings of plant extracts for anti-infective activity due to their reported use in traditional medicinal practices in the Mediterranean for the treatment of infections *(40, 42)*.

Perhaps the most important innovation of all is translating the following phenomenon from traditional medicine into the clinic: while antimicrobial resistance has always developed to single chemicals used in monotherapy, it has not emerged detectably where traditional healers have treated patients with whole plants. One example of this is artemisinin and its parent plant, *A. annua*. It is established for numerous diseases, including malaria, that resistance to two or more drugs administered in combination will develop more slowly. This is because instead of requiring mutations to yield resistance to one mechanism of action, now the pathogen would need to simultaneously develop more mutations to occlude yet other mechanisms of action. For this reason, artemisinin is commonly co-administered with other anti-malarials *(44)*. A recent study showed that oral delivery of the dried leaves of whole plant A. annua overcame existing resistance to artemisinin in a rodent model of malarial infection *(45)*. Moreover, stable resistance to the whole plant took three times longer to develop than stable resistance to artemisinin alone. It may very well be that treatment with whole plants or extracts thereof, which contain hundreds of unique secondary metabolites, represents the most advanced form of combination therapy. Perhaps whole plant or crude extract treatment utilizes the plant's intrinsic multicomponent defense system to make the development of enough resistance mutations statistically unfeasible. Such observations provide compelling rationale to further explore crude plant extracts for the minimal components responsible for yielding the full multi-pronged defence. To this extent, the US FDA has a botanical drug track which accommodates botanical compositions that are well-defined *(46)*.

Conclusions

It is clear that phytochemicals represent a promising source of novel anti-infectives and agents for possibly circumventing antimicrobial resistance. What is more, as a drug discovery resource, phytochemicals remain largely untapped. A recent report analyzing drugs approved by the US FDA has concluded that natural products in general and derivatives thereof have to date contributed much to drug discovery efforts, are likely to continue contributing in the future, and that public and private investment into natural product drug discovery is highly justified *(37)*. There exists much opportunity for innovation based on an understanding of phytochemicals. For instance, a current paradigm in drug discovery is to begin with drug-like synthetic chemicals and sift through them in search of bioactivities. However, considering that phytochemicals represent one of the riches sources of biologically-relevant chemicals, an alternative paradigm to explore is to begin with bioactive plant secondary metabolites and then apply filters of drug-likeness, pharmacokinetics, and so on. With attention now increasingly directed at phytochemicals as a promising source anti-infectives, their re-emergence as a major contributor to this field is but imminent. ■

Figure 1: The ethnobotanical approach to drug discovery. Knowledge of traditional medicinal uses for plants is consulted to identify plants with potential therapeutic phytochemicals. These plants are then collected, their identities are verified, and a specimen of each plant is preserved in an herbarium. Bulk plant material is prepared for chemical extraction, and the extracts are employed in bioactivity screens. Bioactive extracts then go through the process of bioassay-guided fractionation, eventually leading to the isolation of single bioactive phytochemicals. Throughout this process, data is recorded in a database



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