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Current Opinion in Microbiology

Opportunities for plant natural products in infection control

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The continued spread of antimicrobial resistance represents one of the most serious infectious disease threats to global health. There is consensus that a key component of addressing this threat is to replenish the waning pipeline of antimicrobials, with attention being paid to novel mechanisms of action. This includes the development of new classes of classic bacteriostatic and bactericidal antibiotics as well as antivirulence drugs, and it is especially in these areas where plant natural products demonstrate great potential. To this end, we discuss the unique characteristics of plant natural products, the advantages of plants as a resource for anti-infective drug discovery, and recent technologies that have further enabled this path of inquiry. As a result of emerging realization of their advantages, plant natural products have recently enjoyed increased scrutiny in antimicrobial lead discovery, and they will continue to serve as a source of leads. We conclude that plant natural products represent a promising and largely untapped source of new chemical entities from which novel anti-infectives can be discovered.

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Current Opinion in Microbiology 2018, **45**:189–194

This review comes from a themed issue on **Antimicrobials**

Edited by **Gilles van Wezel** and **Gerard Wright**

https://doi.org/10.1016/j.mib.2018.08.004

Introduction

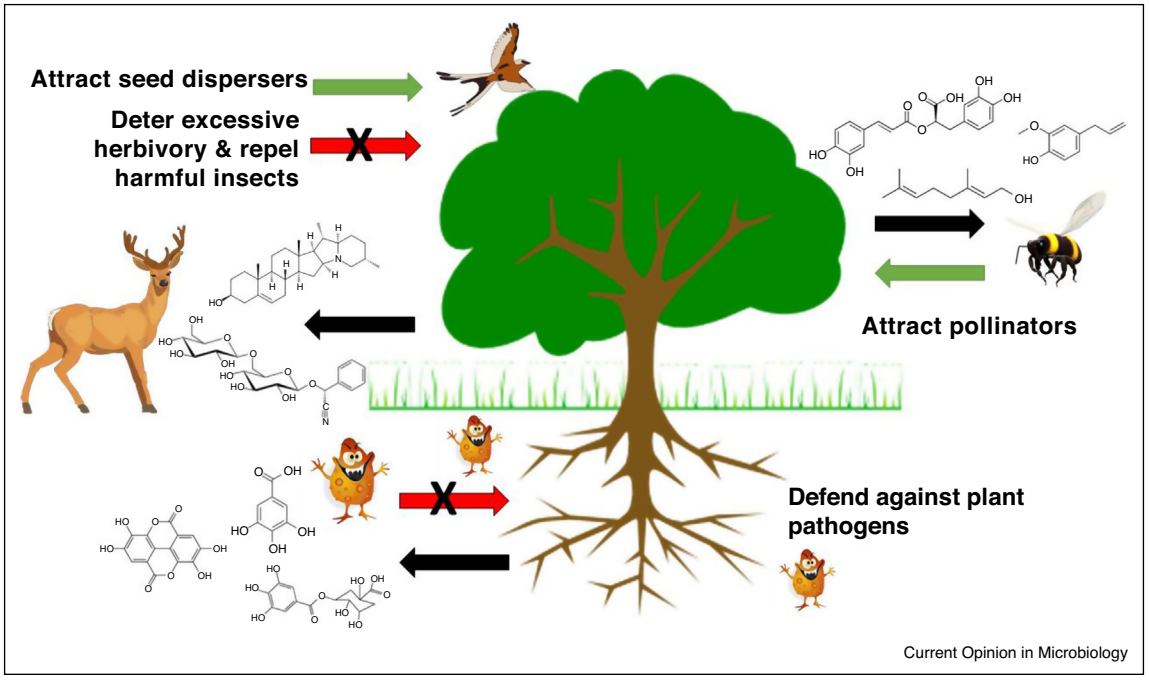
As antimicrobial resistant infections become more and more common, the need for new drugs that circumvent resistance arises as one of the main challenges in combatting this global health phenomenon [1]. Indeed, numerous voices in the literature have cited innovation in anti-infective drug discovery as one of the most important aspects of infection control moving forward [2,3]. This innovation includes the development of drugs that inhibit microbial growth through novel mechanisms of action as well as drugs that work otherwise to attenuate pathogenicity, such as by inhibiting virulence factor production. The latter category of drugs is largely projected to serve as a source of adjuvants to antibiotics that may enhance potency and delay the onset of antimicrobial resistance [4,5•]. This projection has received much attention in the literature, with much *in vivo* evidence supporting the effectiveness of adjuvants in infection treatment [6,7]. Herein we elaborate as to how natural products are especially well-positioned to help fill this gap in the anti-infective pipeline.

Plant secondary metabolites

Most plants produce hundreds if not thousands of unique compounds as an adaptation to their environment for the purpose of self-defense and interaction with other organisms in the environment; these are collectively referred to as plant secondary metabolites, or natural products (Figure 1). The set of total compounds contained in any plant tissue in fact represents a chemical library from which bioactive compounds may be mined [8]. Such libraries possess many characteristics highly favorable for drug discovery. Chief among these is their chemical and structural diversity, which stands in excess of many synthetic small molecule libraries [9]. With this diversity, plant natural products largely belong to the biologically relevant chemical space, which represents the subset of all chemicals that possess bioactivity (Figure 2); they are also largely metabolite-like, thus largely allowing for recognition by transport systems for entry into tissue [2,10–12]. Lead discovery efforts over the last two decades have shifted toward the screening of less structurally complex synthetic compounds, and while there have been many success stories from these campaigns, infectious diseases remain one of the areas that often require chemically and structurally complex molecules [13].

Of the plant natural products explored to date, those tested for microbial growth inhibiton have tended to exhibit weaker potency and selectivity than microbial natural products [14], with some exceptions. Acylphloroglucinols from S. Johns Wort species (*Hypericum* spp.) have demonstrated MICs in the range 0.5–1 μg/mL in methicillin resistant *Staphylococcus aureus* (MRSA) isolates [15••]. At the same time, plant natural products are very clearly rich in anti-virulence properties, with numerous

Figure 1



Plants are sessile and individuals cannot physically move toward resources or away from threats in the environment. Instead, plants produce secondary metabolites — also known as natural products — as chemical communication tools in response to environmental cues. These metabolites are differentiated from the ubiquitous primary metabolites — which include carbohydrates, lipids, proteins, and nucleic acids — and are used for the basic processes involved in maintaining plant life. Secondary metabolites also come at an additional energy cost to the plant, and thus are not produced without reason. Some of the purposes that these compounds serve are in defense against predation and herbivory, attraction of pollinators and seed dispersers, and competition with other organisms in the environment. Secondary metabolites are responsible for the colors, flavors and odors of plant species.

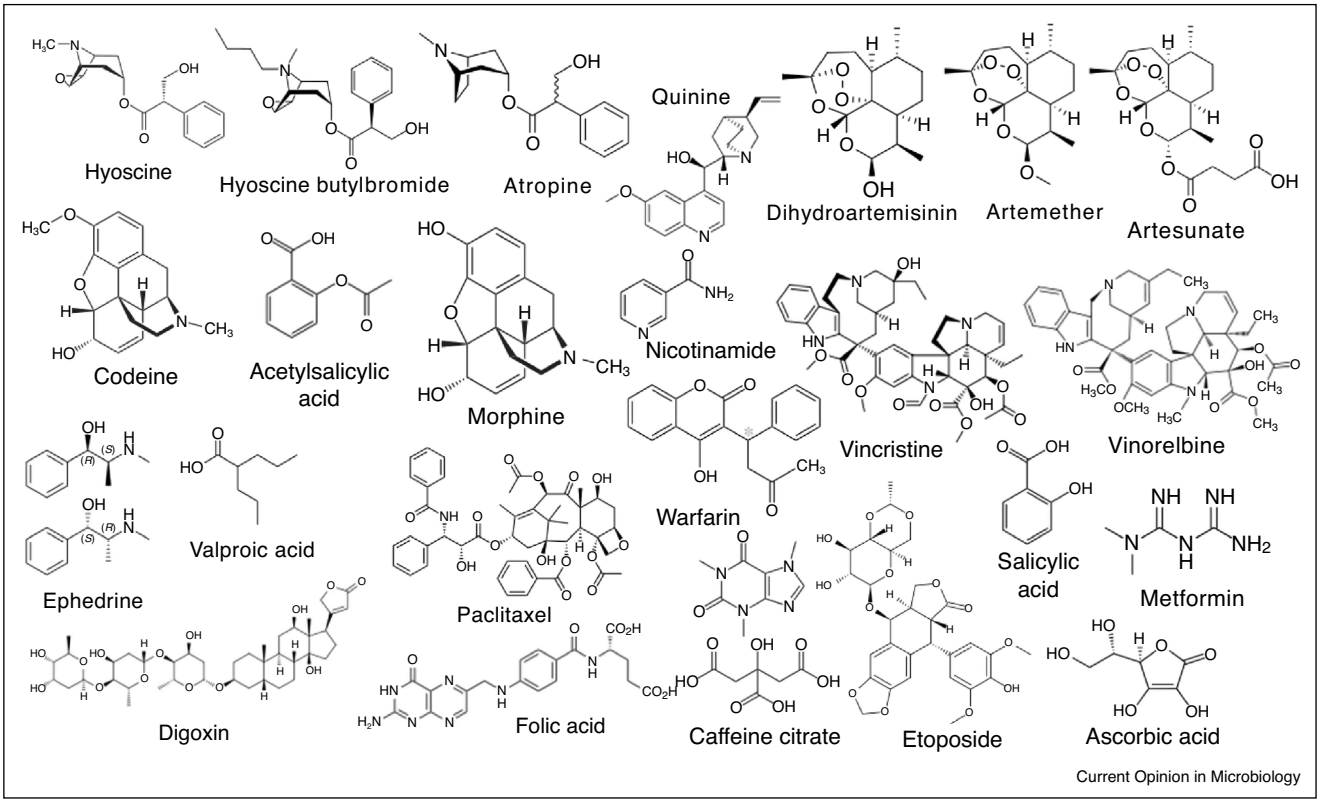
single compounds currently in development to this end [14,16,17]. Epigallocatechin gallate, a major component of green tea catechins, was identified as a promising non-bactericidal antivirulence agent against *Streptococcus pneumoniae* [18•]. Hamamelitannin, a tannin found in the bark and leaves of American witch hazel (*Hamamelis virginiana*), and derivatives thereof are being actively studied for the potentiation of vancomycin in biofilm-associated MRSA infections [19,20••]. INP1855 is a derivative of 8-hydroxyquinoline, synthesized in roots of the diffuse knapweed (*Centaurea diffusa*) and was identified in a screen of a synthetic small molecule library [21,22]. It was confirmed to inhibit the injectisome and flagellar type III secretion systems in *Pseudomonas aeruginosa*, thereby impairing virulence [23••]. There are also examples of synthetic small molecule antivirulence compounds that resemble plant natural product pharmacophores. One example is Compound 22, an isoquinolone mannoside that targets the type 1 pilus adhesin FimH in *Escherichia coli* [24]. Another example is virstatin, an isoquinoline that targets pili biogenesis in *Acinetobacter baumannii* [25,26].

Ethnobotany as a drug discovery tool

In addition to the diversity and drug-like chemical character of plant natural products, the ability to explore this chemical space in a targeted fashion using the lens of ethnobotany — the study of how people use plants — represents a major advantage. This is made possible by the centuries-old practices of traditional medicine in societies across the world, which have identified indications for countless different plant preparations. A recent report on the State of the World's Plants noted that there are at least 28 187 species that have been documented as being used in traditional medicine [27]. While there is no accurate report of how many of these have been investigated to date for their pharmacologic potential, it could be estimated that only a few hundred have been subjected to in depth pharmacologic analysis for bioactivity and chemical composition.

An example of where ethnobotany has guided the discovery of antimicrobial compounds is the immensely successful antimalarial, artemisinin. Malaria is a mosquito-borne infectious disease caused by parasitic protozoans belonging to the *Plasmodium* genus. In 1967, a plant screening research program under the name *Project 523* was set up by the Chinese government with the goal of discovering novel antimalarial chemicals [28]. Tu Youyou was part of a group working on the isolation of antimalarial candidates from plants used in traditional Chinese

Figure 2



What do these compounds share in common? They are all included in the 2017 WHO List of Essential Medicines and they were all originally discovered in — or modeled after chemical scaffolds discovered in — plants. Today, many of these are mass-produced using tools from plant biotechnology and synthetic chemistry.

medicine. Initially, an extract of sweet wormwood (*Artemesia annua*) yielded promising antimalarial activity in a mouse model of infection, though the results were not reproducible. Following preparation for malaria symptoms found in the 4th Century book *The Handbook of Prescriptions for Emergency Treatments* by Ge Hong, Tu Youyou prepared an *A. annua* extract consistently effective in the mouse model of malarial infection. It was through this extraction method that artemisinin was subsequently isolated.

Of the hundreds of compounds contained in different plants, it is small subsets of these compounds, if not single compounds, that are responsible for the therapeutic effects of these plant preparations. As such, by consulting traditional medicinal practices, plants can be identified that potentially contain chemicals of interest for development against a given disease state, and it is this set of plants on which drug discovery efforts can be focused [29]. The identified plants must first be collected and processed for chemical extraction [30]. There are numerous methods of chemical extraction, most of which make use of a solvent as the vehicle through which chemicals are dissolved from the plant material into a liquid phase that can then be separated and dried into a powder [31]. Yet another advantage of plants as a chemical library source is the relative ease of generating large quantities of chemicals, which only requires collecting and processing more plant material. The dried plant extracts are then subject to the drug discovery framework of bioassay-guided fractionation, in which bioactive compounds are isolated by iterative rounds of chromatographic fractionation followed by assessment of biological activities of the fractions. Once natural products of interest are identified, more efficient chromatographic methods may be developed for their isolation.

Synergy among natural products

Another advantage of plant natural products for infection control is the potential for discovering highly effective synergies between different compounds in a given plant extract [32]. Such synergies may lead to increased efficacy and a diminished tendency for the evolution of resistance [33]. Artemisinin and its plant of origin, *A. annua*, can also serve as an example that illustrates this potential. In a rodent model of malarial infection, oral delivery of the plant's dried leaves was compared to treatment with a dose of pure artemisinin matching the whole plant

content [34]. The dried leaf treatment was found not only to be more efficacious at attenuating parasitemia, but also to result in a 40-fold increase in artemisinin bioavailability in the rodents. A later study, also in a rodent model of malarial infection, showed both that oral delivery of the dried leaves overcame existing resistance to artemisinin and that stable resistance to the whole plant treatment took three times longer to develop than stable resistance to artemisinin alone [35]. Although the mechanism of action is not known, it could be that other chemicals present in the whole leaves target at least one other pathway important for the plasmodial life cycle, making the development of a resistant strain less likely to occur.

Indeed, there are a number of examples in the literature where multiple metabolites in the same plant have been identified and shown to synergistically exert a pharmacological effect. For instance, through synergy-directed fractionation, three flavonoids from goldenseal (*Hydrastis canadensis*) were identified, each of which capable of enhancing the antimicrobial efficacy of berberine, also present in *H. canadensis* [36]. It was shown that all three flavonoids possessed no inherent antimicrobial activity against *S. aureus*, but rather functioned as inhibitors of the NorA multidrug resistance pump. With such multi-component defense systems being identified in plants, the possibility of developing plant natural product fractions that exhibit more favorable activity and resistance profiles is highly attractive. To accommodate such botanical compositions, the United States Food and Drug Administration (US FDA) has a botanical drug track that requires them to be well-characterized [37]. Natural product synergies can be highly complex and difficult to define, and we will discuss avenues for deconvoluting such complexities.

Drug discovery from plant natural products

Performing classical drug discovery from plant natural products is more accessible than ever before. In decades past, natural products in general had received diminished attention in favor of combinatorial and synthetic chemical libraries. More recently, difficulties that would have hindered plant natural products research have been overcome, such as improved compatibility with high throughput screening (HTS) and improvements in foreign plant acquisition, dereplication, compound isolation, and medicinal chemistry [2,9,38,39]. Moving forward, the field of metabolomics stands as one of the strongest enablers of plant natural product discovery, allowing scientists to effectively mine the extremely chemical diversity available [40••].

Metabolomics transforms the classical framework of bioassay-guided fractionation into a much shorter process of rapid identification of valuable plant natural products. In its most general sense, metabolomics is the analysis of all the known and/or unknown metabolites in a given biological sample. From such a dataset, very complex analyses can be undertaken to ask questions such as 'which pharmacophores tend to be present in the most bioactive fractions of this extract' and 'which compounds are necessary for any possible synergism in fractions with the best resistance profiles' [41].

For metabolomics studies in plant natural product drug discovery, the acquisition of high quality data from a sample has been facilitated by technological advancements in chromatography, which separates the metabolites of a sample over time, and spectroscopy, which provides chemical data on said metabolites [42]. Liquid chromatography–mass spectrometry (LC–MS) represents the most useful platform for profiling natural product compositions, and high-quality data is particularly achievable with ultra-performance liquid chromatography–high-resolution mass spectrometry (UPLC–HRMS) [43,44].

From MS data, molecular features (molecular ions, adducts, in-source fragments, etc.) can be grouped and putative molecular formulae of metabolites can be determined [40••]. An MS data organization tool can then use MS fragmentation (MS/MS) data to link metabolites based on attributes such as similarity in spectrum or structure or presence of a particular structural moiety. More layers of information can then be mapped to the organized data, such as bioactivities, taxonomy, and gene sequences. This way, the MS/MS data can be organized as a molecular network of features that can then be annotated, ideally by unambiguously linking unique identifiers to a single molecular structure. Once a molecular network is annotated, it becomes possible to apply different types of scoring to refine the annotation results. As such, valuable natural products are identified from plant extracts or fractions thereof while at the same time massive metadata sets are constructed for these samples, which can be mined under various criteria. For example, such a metabolomics approach was used to focus isolation efforts on previously undescribed compounds from the leaves of *Palicourea sessilis* [45••]. An alkaloid fraction of the leaves was analyzed, and MS/MS data were organized as molecular networks [46]. The metabolites were then grouped in clusters of similar scaffolds, and the data was annotated against *in silico* spectra of natural products present in the *Dictionary of Natural Products* [47•]. Following this, compounds that did not match any known natural products were not annotated, indicating the presence of novel compounds.

Success in anti-virulence approaches

Demonstrating the strong potential for plant natural products in anti-infective drug discovery is the sheer volume of isolated compounds with confirmed anti-virulence activities, covered in recent review articles [16,17]. The state of the literature truly demonstrates that anti-virulence is the main category of bioactivity exerted by plant natural products, and that a vast number of different pharmacophores have been identified in this domain.

This is the case, even though plants as a source of chemical diversity remain largely untapped [48]. Only an estimated 15% of approximately 300 000 higher plant species have been investigated phytochemically, and only a nominal fraction of these has been investigated for anti-infective potential [49]. As metabolomics has yet to enjoy widespread use in plant natural product drug discovery laboratories across the world, the promise for infection control seems even greater. Additionally, advances in bioreactor technology and metabolic engineering have and continue to improve the production, manipulation, and scientific understanding of both single molecule and synergistic mixtures of therapeutic plant natural products [29]. These advances have culminated in the birth of ethnophytotechnology, defined as 'the use of plant biotechnology to improve or enhance the inherent economic or culturally valuable traits of plants as described and influenced by ethnobotany [29].'

Conclusions

We have summarized the advantages and potential that plant natural products present for anti-infective drug discovery. The field is in need of novel pharmacophores, and to this end, plant natural products represent an underinvestigated region of the chemical space where targeted exploration can be undertaken with the aid of new technologies such as metabolomics. It is important to note that while plant natural products hold such potential, synthetic approaches are being increasingly utilized to construct chemical scaffolds bearing the complexity of natural products, traversing into regions of the chemical space beyond the scope of nature [50•]. Within the realm of what nature can synthesize, microbial and marine natural products will continue to be studied for new chemical entities. It is ultimately this expansion into the yet underexplored chemical space where plant natural products will play a unique and critical role in the discovery of the next generation of anti-infectives.

Conflicts of interest statement

Nothing declared.

Acknowledgements

Work in the Quave Research Group is funded by the National Institutes of Health, National Institute of Allergy and Infectious Disease (R21 AI136563, PI: CLQ). The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH or NIAID. The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Our sincere apologies to authors whose publications could not be cited due to the article length constraints.

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