Enlisting plants in the battle for new antibacterial compounds

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A rise in antibacterial drug resistance comes at a time when our once reliable sources of antibacterial natural products, bacteria and fungi, are failing us. The search for new drugs to fight pathogens has led to a range of innovative approaches and includes screening organisms which have developed evolutionary adaptions to prevent bacterial attack. The discovery of antibacterial phytochemicals from plants can be achieved using an activity-guided platform involving biological and chemical pre-screening, compound isolation, structure elucidation, and the direct testing of isolated compounds. Challenges include the clean isolation of natural products, avoiding the rediscovery of known compounds, toxicity, and poor levels of activity.

For a good part of the 20th century, humans had the upper hand against bacterial pathogens thanks to the pioneering work of Alexander Fleming, René Dubos and Selman Waksman *et al.* who demonstrated the value of mining antibacterial natural products from bacteria and fungi. For a few decades this approach (which yielded the likes of penicillin, streptomycin and tetracycline) seemed to be an impenetrable fortress against bacterial pathogens, until the walls began to strain under the force of growing antimicrobial resistance and a dearth of new antibiotic classes[1]. The 'old' approach eventually failed to produce significantly new clinical agents against the background of known compounds[1]. Researchers have responded in a range of novel ways: running large compound libraries through high throughput screenings[2], mining the natural products in previously unculturable organisms[3], screening the chemicals hidden away on the shelves of chemistry labs[4], disarming bacteria of their virulence factors[5], and developing phage therapies[6]; each approach with its merits and limitations. Another approach is screening botanical natural products.

The plant world as a whole is estimated to produce over 100 000 secondary metabolites with low molecular mass, generally derived from isoprenoid, phenylpropanoid, alkaloid and fatty acid or polyketide pathways[7]. While plants and animals have some common antibacterial defences such as apoptosis of infected tissue, antibacterial peptides (purothionins from *Triticum aestivum*[8] are a noteworthy plant-based example) and the targeted exploitation of reactive oxygen species, they do not produce antibodies, relying instead on a limited number of receptors to recognize pathogens along with a diverse armoury of small molecules with antibacterial activity[9]. Compounds with known specific antibacterial targets are not common in plants, although there are examples such as coumarins with comparable action to the DNA-gyrase inhibitor novobiocin[9]. While activity is usually weak, it is possible that plants target virulence rather than growth or that relatively weak antibacterial agents work in synergy with each other to create potent activity as seen with the antibacterial compound berberine from *Berberis fremontii* together with the multi drug resistance (MDR) pump inhibitor 5'-methoxyhydnocarpin[9,10].

Important phytochemical groups include phenolics and polyphenols, quinones, coumarins, flavonoids, terpenoids and alkaloids[11] (Figure 1). Phenolics and polyphenols include the simple phenols, phenolic acids and tannins. Antibacterial examples are found in the tea plant *Camellia sinensis* and include gallic acid, a phenolic acid which disrupts cell membranes[12,13], and the tannin tannic acid which reduces *Staphylococcus aureus* biofilm formation[14]. A representative of the quinones is juglone found in the black walnut tree *Juglans nigra*[15], while the coumarins include osthole found in *Arracacia tolucensis* var. *multifida*[16]. Flavonoids include myricetin, found in the sweet potato plant *Ipomoea batatas* and which appears to affect protein synthesis[17,18].

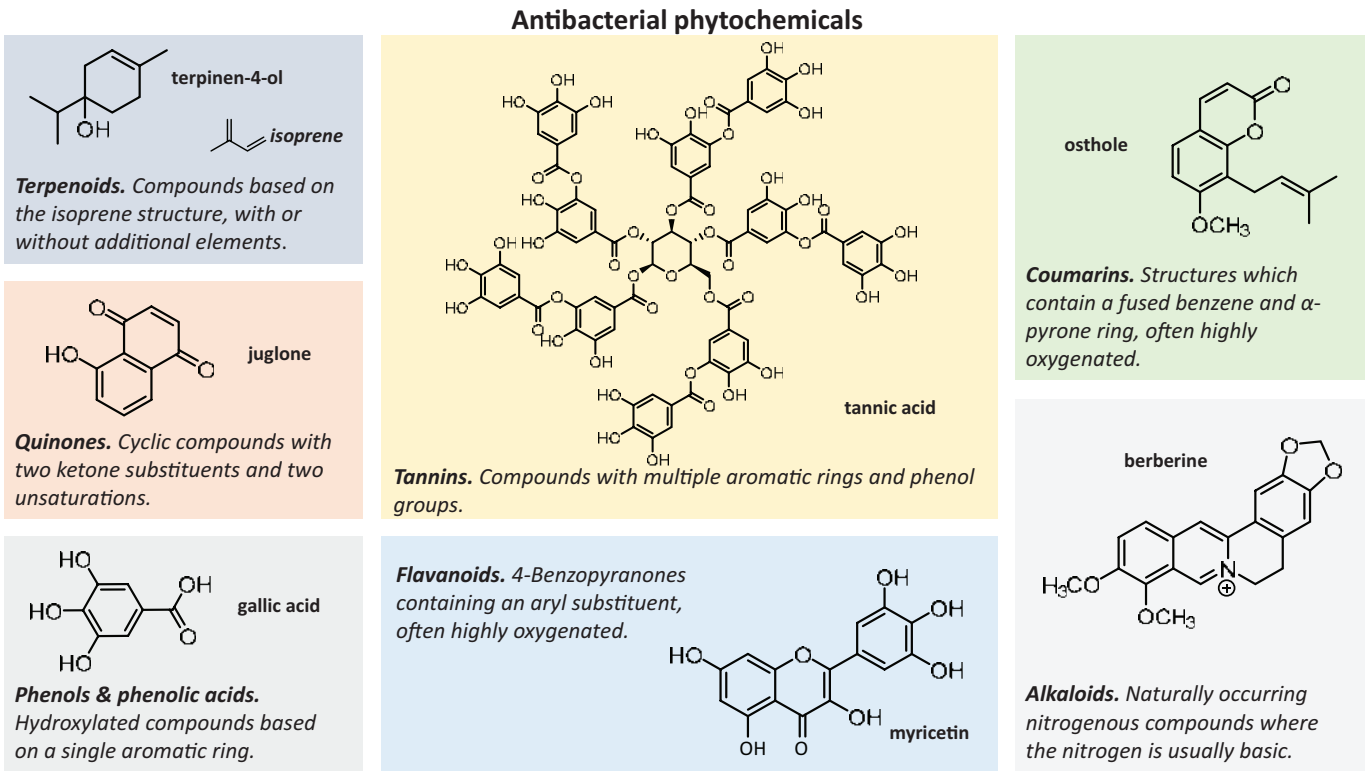

Figure 1. Examples of antibacterial phytochemicals and the chemical classes to which they belong.

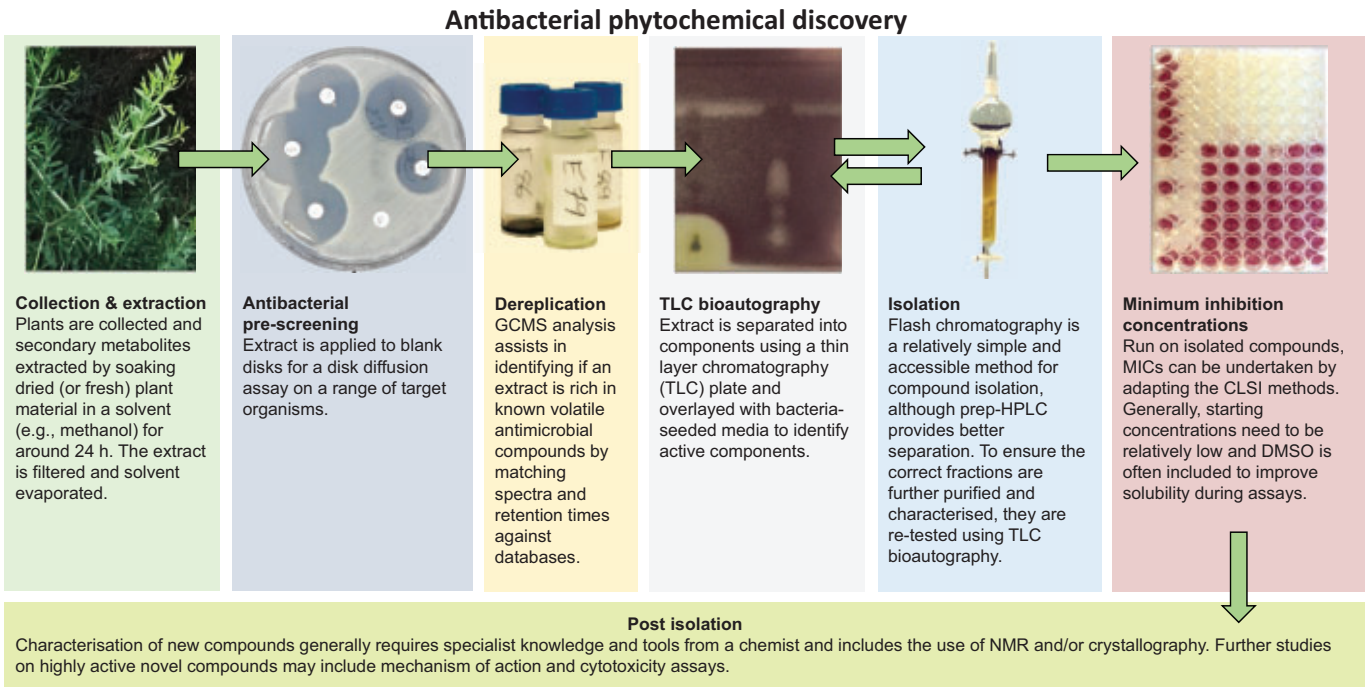

Figure 2. Schema for discovering antibacterial phytochemicals.

Terpenoids are common phytochemicals based on the isoprene structure and include terpinen-4-ol, an antibacterial terpene found abundantly in *Melaleuca alternifolia* tea tree oil[19], which evidence suggests leads to damage to the cell membrane and loss of cytoplasmic material[20]. Alkaloids are another common phytochemical group and include berberine (previously discussed) found in *Coptis chinensis* and *Berberis fremontii*. The mode of action of berberine may be through binding with double helical DNA[21] and/or the inhibition of the bacterial division protein FtsZ[22].

There are many ways to decipher a plant's defences to find these antibacterial small molecules and these are accessible to a microbiologist who has support from a multidisciplinary team that includes a chemist and botanist. A simple schema developed in our lab is shown in Figure 2 and involves plant collection, secondary

metabolite extraction, antimicrobial disk diffusion screen (adapted from the EUCAST[23] method), GC-MS analysis coupled with databases (e.g. NIST Mass Spectral Libraries) for dereplication, TLC bioautography[24,25], compound isolation by flash chromatography (or prep-HPLC) guided by testing fractions by TLC bioautography, elucidating new compound structures by NMR and/or crystallography and undertaking MICs on isolated compounds (adapting the CLSI methods[26]). Additionally, screening phytochemicals against specific virulence factors could uncover a trove of treasures, but there are diverse targets[5] and each target requires a suitable assay: ultimately lots of work which may result in few if any hits. Inclusion in a compound library for high throughput screening is a possible solution. If good activity is seen during crude extract screening but is poor in the isolated compounds, combinations of compounds suspected to potentiate each other can be tested in a checkerboard assay[27].

While the potential of antimicrobial phytochemicals is clear, there is a dearth of examples that have made it into the clinic. Many reasons for this exist including the differences in human and plant biology and physiology giving rise to toxicity concerns. An isolated compound with promising MIC activity needs to demonstrate low toxicity with preliminary tests such as *in vitro* cytotoxicity assays[28] presenting a hurdle. Other factors for the lack of plant-based antibacterial agents include plants making diverse antimicrobial compounds but each with relatively poor activity[9], and their production of a range of structurally similar compounds making isolation difficult and resource intensive. Compounding these problems, often the researcher spends time and resources to simply discover a known compound.

Attacking drug resistant bacteria from multiple fronts gives us the best chance for success. Screening phytochemicals as one of those approaches makes sense given the reliance of flora on secondary metabolites for antibacterial protection, and the incredible diversity of structures present across an enormous number of plant species. While plants have thus far generated few clinical candidates, successes in other anti-infective classes such as that of the antimalarial drug artemisinin from *Artemisia annua*[29] allow for optimism. In Australia, only a limited number of researchers have looked at our unique flora as a potential solution and research has tended to focus on a limited number of genera, notably, *Acacia*, *Melaleuca*, *Eucalyptus* and *Eremophila*, leaving most species still to be screened.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

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