Accelerated approach of discovering plant derived drug leads for treatment of TB

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Natural products are a major source of novel drug lead compounds due to their unparalleled structural diversity. The classical approach of bioassay-guided fractionation to target the active constituents in plant extracts is time consuming, often leads to the re-discovery of known compounds and loss of activity in the course of the purification process is not uncommon as the process may neglect interesting compounds with minor biological activity. These secondary active compounds discarded during the traditional approach may contribute to the overall activity of the extracts and present interesting chemical scaffolds that could make them preferential candidates for their chemical optimisation into therapeutic candidates. More targeted approaches are needed in order to identify promising candidate molecules upfront and accelerate the development process.

An accelerated drug discovery approach based on 96-well fractionation and LC-MS-MS de-replication technology has been implemented at CSIR Biosciences for:

- accelerating the identification of active ingredients and interesting chemical scaffolds for subsequent medicinal chemistry developments from medicinal plants
- accelerating the scientific validation of traditional medicines and providing Traditional Healers with chemical and biological information
- optimising the biological annotation of natural compounds contained within botanical extracts
- preventing the repeated discovery of known compounds thereby saving time and resources
- accelerating structure elucidation of novel compounds
- flagging of cytotoxic compounds

The research conducted by the Bioprospecting group to identify TB drug leads using the accelerated approach will be outlined. The Bioprospecting platform in collaboration with the South African National Botanical Institute (SANBI) has established a dedicated and comprehensive plant electronic database of a total of 566 plants that are reportedly used for the treatment of tuberculosis. The extracts of these plants are part of the CSIR database of extracts. A process of prioritization using selection criteria led to 228 plant extracts being tested in a preliminary *in vitro* screen against *Mycobacterium aurum.* Thirteen organic (1:1 methanol: dichloromethane) extracts that demonstrated significant antibacterial activity, i.e. Minimum Inhibitory Concentration (MIC) ≤ 1000µg/ml, were tested against *M. tb.* and resulted in four biologically active extracts (MIC ≤ 250µg/ml).

The extracts displaying good anti-tuberculosis activity were fractionated using semi-preparative HPLC and the fractions were screened for anti-tuberculosis activity. The active fractions were analysed by UPLC-TOF-UV-MS for UV, accurate mass and MS-MS fragmentation data to identify the compounds present. By using this data to search the Dictionary of Natural Products scientists could determine the classes of compounds responsible for activity and could make upfront go/ no go decisions depending on the compounds desirability as drug leads. The approach to accelerate the identification of potentially new anti-TB leads has proved successful as de-replication has lead to the identification of some compounds that have either been previously reported for use or are of the biologically undesirable class of compounds. TB drug lead research is ongoing, and active ingredients will be subjected to computational chemistry to identify potential sites for structural modification to improve bioavailability and decrease cytotoxicity.