South African plants as a source of drugs to treat infectious diseases - TB, Malaria and HIV

P. PILLAY*, D. NAIDOO*, V. MAHARAJ, N. MOODLEY, P. SEWNARAIN, S. VAN ROOYEN, X. MTHEMBU, E. KHOROMBI

*CSIR Biosciences, PO Box 395, Pretoria, 0001
Email: PPillay@csir.co.za, DNaidoo2@csir.co.za

Abstract

CSIR Biosciences is actively involved in identifying new medicines effective against tuberculosis (TB), malaria and HIV based on South Africa’s rich biodiversity.

As part of a national multidisciplinary consortium, the Bioprospecting research group and the South African National Biodiversity Institute (SANBI) established a database of 566 plant taxa that are reportedly used for the treatment of TB and 623 taxa associated with malaria and/or fever. A process of prioritization using selection criteria led to 162 plant extracts, representing 24 taxa, being tested in a preliminary in vitro screen against Mycobacterium aurum. Thirteen extracts demonstrated significant antibacterial activity and were subsequently tested against M. tuberculosis. This led to 7 extracts (5 taxa) with significant anti-TB activity. Of the 134 plant taxa tested for in vitro antimalarial activity against a chloroquine-sensitive strain of Plasmodium falciparum, 23 species were found to be highly active. Bioprospecting has screened 30 plants with traditional use related to the treatment of HIV for their anti-HIV properties, resulting in the identification of four biologically active plant extracts (4 taxa), which are currently being further developed. A recent research collaboration with Esperanza Medicines Foundation (EMF) also provided a unique opportunity to evaluate South African medicinal plants in HIV/AIDS-related biological assays exclusive to the Swiss-based institute.

Overall, compelling evidence has been provided for the rational exploration of South African plants as sources of new drugs to treat TB, malaria and HIV.

1. Introduction

South Africa boasts a rich plant biodiversity of approximately 24 000 indigenous plant species that not only offers a physical attractiveness but also provides a wide spectrum of uses including the medicinal properties that have been used for generations by indigenous people to treat various ailments and diseases. The Bioprospecting research group at CSIR Biosciences undertakes research that taps into these natural resources with the aim of discovering natural products that can be used to develop new medicines effective against tuberculosis (TB), malaria and HIV.

The burden of disease and mortality in South Africa (TB and HIV) and on the African continent (TB, malaria and HIV) are amongst the highest in the world. With the rapid increase in infection in the sub-Saharan region and due to the relatively high cost and limited access to synthetically derived drugs, communities in Africa have relied on traditional methods to treat infectious diseases (WHO, 2003). These unconventional treatments, often made up of highly complex plant based mixtures, cannot be ignored or dismissed by scientists around the world who are looking for new treatments for these diseases.

The Bioprospecting research group and SANBI, as part of a multidisciplinary consortium, has established a dedicated and comprehensive plant electronic database of a total of 566 plants that are reportedly used for the treatment of tuberculosis and 623 plants associated with malaria and/or fever occurring indigenously or naturalised in the Flora of Southern Africa (FSA) region. Biosciences has over the years also received several “claims for cures” on plants used for the treatment of HIV. In addition to these claims, an extensive literature study was undertaken to identify potential plants used traditionally for the treatment of HIV and opportunistic infections, resulting in a list of 60 plants species.

2. Results and Discussion

Plants were prioritised for screening in relevant biological assays based on weighted criteria (primarily ethnobotanical and chemotaxonomic). Extracts of most of these plants are part of CSIR’s historical repository of extracts and those
that were not, were subsequently collected and extracted.

Due to the slow growth rate and the highly infectious nature of *Mycobacterium tb.*, a strain of the rapidly growing non-pathogenic *Mycobacterium aurum* is currently used as a preliminary anti-TB screen, since it is reported that inhibition of *M. aurum* growth is highly predictive of activity against *M. tb.* (Chung et al., 1995). Hundred and sixty two plant extracts (24 taxa) were prioritised and tested in vitro against *M. aurum* as a preliminary screen. Thirteen organic (1:1 methanol:dichloromethane) extracts that demonstrated significant antibacterial activity, ie. Minimum Inhibitory Concentration (MIC) ≤ 1000µg/ml, were tested against *M. tb.* resulting in seven biologically active extracts (5 taxa) (MIC ≤ 250µg/ml) (Table 1).

**Table 1: The antimycobacterial activity of plant extracts against *M. aurum* A+ and *M. tb*.

<table>
<thead>
<tr>
<th>Extract number</th>
<th>Plant part</th>
<th><em>M. Aurum</em> MIC (µg/ml)</th>
<th><em>M. tb.</em> MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P10433B</td>
<td>roots</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>P07947B</td>
<td>roots</td>
<td>375</td>
<td>250</td>
</tr>
<tr>
<td>P07948B</td>
<td>stems</td>
<td>1000</td>
<td>250</td>
</tr>
<tr>
<td>P07949B</td>
<td>leaves</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>P04413B</td>
<td>leaves</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>P05588B</td>
<td>roots</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>THC020187B</td>
<td>whole plant</td>
<td>480</td>
<td>62.5</td>
</tr>
</tbody>
</table>

MIC ≤ 1000µg/ml is significant for *M. aurum*
MIC ≤ 250µg/ml is significant for *M. tb.*

The extracts displaying antituberculosis activity were subjected to bioassay-guided fractionation to isolate the active ingredients using chromatographic separation procedures. Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry analyses were used to elucidate the structures of compounds present in the active extracts.

Extract P10433B, prepared from the plant *Zanthoxylum davyi*, was previously reported to contain alkaloids (Tarus et al., 2006). Bioassay-guided fractionation of the extract using *M. aurum* led to an active compound, chelerythrine (1) which has previously been reported to have antimycobacterial activity.

(1) P07947B, P07948B and P07949B are extracts of different plant parts of the same plant species. Bioassay-guided fractionation to isolate the active/s are ongoing. The class of compounds present in the extracts were identified as alkaloids.

Extract P04413B was fractionated using semi-preparative HPLC and has been previously reported to contain carbazole alkaloids. The fractions together with a pure compound were screened against *M. aurum*. The screening results showed that activity was lost during the fractionation process.

The isolation of the active ingredients in extract P05588B is currently ongoing.

THC020187B has shown the best activity against *M. tb* as compared to all the extracts screened by our group and the most potent fraction, obtained through chromatography, is currently being further separated to isolate the active/s, also belonging to the alkaloid class of compounds.

The results present an interesting observation as activity of the extracts P10433B and P07949B between *M. aurum* and *M. tb.* were the same, while the other extracts show different MIC values for the two strains (P04413B and P05588B show 2 times more potency, P07947B shows 1.5 times more potency, P07948B shows 4 times more potency and THC020187B shows about 7 times more potency against *M. tb.* relative to *M. aurum*).

The differences could be attributed to the fact that the chemical composition of extracts derived from plants vary between species. Certain classes of compounds may be more active against *M. aurum* and less active or inactive against *M. tb.* and vice versa. It is clear from the results that *M. aurum* is not necessarily the most appropriate organism as we have seen that certain extracts are more sensitive towards *M. tb.* Hence during the bioassay guided fractionation process to isolate the active ingredients, the fractions generated from these extracts will be screened against *M. tb*.

Extracts of plants prioritised for antimalarial screening were tested for in vitro activity against a *Plasmodium falciparum* strain D10 using the parasite lactate dehydrogenase (pLDH) assay. Of the 134 species assayed, 49% showed promising antiplasmodial activity with IC₅₀ (concentration that inhibits parasite growth by 50%) ≤ 10 µg/ml,
while 17% were found to be highly active (IC$\text{}_{50} \leq 5 \, \mu\text{g/ml})$. Several plant species and genera were shown for the first time to possess in vitro antiplasmodial activity (Clarkson et al., 2003).

Significantly active extracts (IC$\text{}_{50} \leq 5 \, \mu\text{g/ml})$ were subsequently screened against a chloroquine-resistant K1 strain and prioritised for bioassay-guided fractionation based on results of activity and literature studies. Some extracts lost activity during bioassay-guided fractionation, possibly due to instability, loss of synergism or lack of reproducibility while others led to isolation of known actives and to compounds with good antimalarial activity (IC$\text{}_{50} \leq 1 \, \mu\text{g/ml})$ but limited selectivity (bioactivity vs cytotoxicity) (Pillay et al., 2007a;b). At least two compounds isolated from active extracts are undergoing further development as potential antimalarials with funding leveraged from the Innovation Fund’s Novel Drug Discovery Platform (IF NDDP) and the European Union’s Antimalarial Programme (EU ANTIMAL).

About 30 plants reportedly being used traditionally to treat HIV were evaluated by screening the relevant plant extracts in a HIV cell-based XTT cytoprotection assay (which quantitatively measures HIV cytopathic effects as well as cytotoxic effects to human host cells allowing the estimation of an in vitro “therapeutic index”). This resulted in 4 hits (4 taxa) being selected for further development as treatment for HIV, based on their traditional use and early biological data. One of these has reached a stage whereby the active ingredients have been identified, with their mode of action currently being determined. The product is being developed as a herbal medicine and a process for the manufacture of the herbal substance has been developed.

In 2007/8 a research collaboration agreement was established between CSIR and the Swiss-based Esperanza Medicine Foundation. The agreements allows for the screening of plant extracts that have been reportedly used for the treatment of HIV and provides access to a battery of HIV assays. These are based on cellular assays for viral replication HIV deCIPhR system (including various subtypes with relevance for Africa such as subtypes D, D/C, A/E, and B, and HIV variants using either of the dominant cellular co-receptor types for HIV: CCR5 and CXCR4, or dual-tropic), combined with assays for cellular cytotoxicity that define a useful cytotherapeutic window.

For this collaborative project a list of 60 plants was identified based on their traditional use for HIV and opportunistic infections. Plants were prioritised for screening at Esperanza based on their history of use, literature precedent, availability, and plant part used. Of the 27 samples initially selected, 22 were evaluated for their HIV activity using the cellular infection system deCIPhR. Fifteen extracts showed consistent reduction of one or both virus types. Nevertheless, their cytotherapeutic window was shown to be relatively limited. In summary, none of the 27 natural substances demonstrated specific antiretroviral activity in concentration range that would not also significantly affect cell viability. A more recent batch of 26 extracts, representing 14 taxa, yielded at least 3 hits with reasonable cytotherapeutic windows, and these are being investigated to isolate and identify the active ingredients.

3. Conclusions

By using a selection of relevant biological assays it has been possible to evaluate the potential of selected South African medicinal plants to treat the infectious diseases – TB, malaria and HIV.

The conventional process for screening extracts for their anti-TB properties is to screen against $M. \text{aurum}$ followed by the slower-growing and more costly $M. \text{tb}$ assays. Our results have shown that for certain plant species, differences are observed between the two organisms and that care should be taken during prioritization of the hits for further development.

The majority of compounds identified thus far have either been previously reported for use or are of a biologically undesirable class of compounds. However, there are still a few promising leads which are undergoing further development, and where active ingredients are identified, these will be subjected to computational chemistry to identify potential sites for structural modification to improve potential problems associated with cytotoxicity and bioavailability.

The evaluation of plants reportedly used for treatment of HIV is a complex one as traditional usage may be based on the treatment of opportunistic infections of the disease, or the plants may have an immunomodulatory effect. As a consequence the extracts tested for their anti-HIV activity will also be tested for their antimicrobial and antifungal properties.
Overall the results of the studies conducted to date confirm the potential of South African medicinal plants in drug discovery and identified a selection of promising taxa and compounds for further investigation as plant based anti-TB, antimalarial and anti-HIV agents.

4. References


5. Acknowledgments

We acknowledge SANBI for the establishment of plant taxa databases and the identification and collection of selected plants.

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6. Author Contributions

Dashnie Naidoo was responsible for the TB research and contribution to this paper, while Pamisha Pillay was responsible for the malaria research, and the malaria and HIV contributions to this paper. V. Maharaj, N. Moodley, P. Sewnarain, S. van Rooyen, X. Mthembu and E. Khorombi contributed to the research results reported here.