Ten years of bioprospecting activities at the CSIR: *BP4 as a model*

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Abstract

The bioprospecting group of CSIR Biosciences, focuses on the transformation of African traditional medicines into minimally-processed, scientifically-validated herbal medicines and remedies; the discovery of new pharmaceutical active ingredients and providing opportunities for the establishment of community-based agro-processing businesses for the production of medicinal crops. This value addition to biodiversity and indigenous knowledge through scientific innovation is conducted through consortium-based research within South Africa. An insightful and strategic look at our results so far reveals a very interesting and valuable data set that immediately provides scientific evidence demonstrating efficacy required for the validation of the claims of traditional healers.

To date, we received and captured more than 250 claims for cures based on medicinal plants; completed desktop/literature studies on at least 50% of these for the purpose of determining what prior research is already in the public domain; established the therapeutic area and identified possible biological assays; identified at least 80 claims for cures for which the therapeutic concepts were established for different diseases, e.g. asthma, arthritis, malaria and HIV; and tested samples for at least 33 claims for cures in suitable biological assays of which 16 demonstrated positive results and are in further development (referred to as bioprospecting leads). Currently, these leads are in development for treatment of cancer, asthma and allergies, arthritis, inflammation, benign prostatic hyperplasia, malaria, HIV, erectile dysfunction, pain, tuberculosis (TB) and diabetes.

One of the leads currently being developed is BP4, a novel herbal extract from *Siphonochilus aethiopicus* for the treatment of asthma and allergies. Literature studies on the specific plant provided anecdotal information but little scientifically evaluated biological data. Biological assaying of extracts of the plant and a purified non-steroidal metabolite demonstrated efficacy in the glucocorticoid receptor binding, inhibition of 5-lipoxygenase, and phosphodiesterase 4 enzymes. The extract demonstrated reduced infiltration of inflammatory cells in the lung tissue, as well as decreased eosinophils in the bronchiolar lavage fluid in asthmatic mice which was comparative to dexamethasone, a corticosteroid used for the treatment of asthma. The results demonstrated the anti-allergic properties of the plant extract providing scientific evidence which substantiates its traditional use.

1. Introduction

Biosprospecting is the search and sustainable use of chemical and genetic components of biodiversity and indigenous knowledge (IK) that can lead to the creation of economic and social benefit for the nation and the region. South Africa is considered to be a ‘hotspot’ for biodiversity and more than 24 000 plant species occur within its boundaries. This represents 10% of the world’s species, although the land surface of South Africa is less than 1% of the earth [1]. The country is divided into seven biomes and 68 vegetation types [2]. The savanna biome covers 33% of the surface of the country, but it is especially the Flora Capensis that is unique. This, the Cape Floral Kingdom, is the smallest of the world’s six floral kingdoms. It contains 8 700 species of which 68% are endemic.

Indigenous medicinal plants are used by more than 70% of South Africans in their healthcare needs or cultural practices [3]. Approximately 3 000 species are used by an estimated 200 000 indigenous traditional health practitioners (THPs) [4]. THPs in South Africa play a crucial role in providing healthcare to the majority of the population. They are the first healthcare providers to be consulted in most cases, especially in rural areas, and deeply interwoven into the fabric of cultural and spiritual life.

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community-based agro-processing businesses for the production of medicinal crops.

Drug discovery based on indigenous knowledge (IK) and biodiversity is an interactive process involving discrete stages. The research approach begins with capturing of IK (claims for cures) on the use of medicinal plants and establishing the disease/ailment being treated leading up to a therapeutic concept. Following the botanical classification and an intensive literature study in trying to establish published information on the medicinal plant, extracts are prepared using conventional organic chemistry approaches as well as a laboratory process that mimics the preparation used by the traditional healer. The resulting extracts are tested in suitable in vitro or in vivo biological models once these are identified and sourced. Non-target based assays are preferred as traditional medicines often act through novel targets and have unique modes of action, e.g. testing against whole parasites such as Plasmodium falciparum for plants used to treat malaria.

Once efficacy is demonstrated, it is regarded as 'a hit' and often provides the first scientific evidence that substantiates the traditional use. The next stage is the isolation and identification of the active ingredient responsible for the activity. This stage often defines the nature of the 'lead' as to whether it is a single chemical entity or quite frequently a botanical extract due to synergistic effects of a complex mixture containing several chemical constituents. Leads based on single chemical compounds frequently require optimisation using synthetic modification to improve potency while decreasing toxicity. Early mode of action studies is also included at this stage. The optimisation of complex botanical mixtures typically require processing and product development including cultivation and harvesting procedures. The research process established by the group is shown in Figure 1.

2. Results and discussion

Protection of intellectual property rights (IPR)

THPs currently provide scientists with IK that stimulates research that could eventually allow the filing of patent applications. The right of the providers of IK to share in future financial benefits that might be derived from commercial exploitation of any such future patented inventions, is protected through a Memorandum of Understanding (MoU) (September 1999) and a Benefit Sharing Agreement (BSA) (February 2003) signed between the CSIR and a Traditional Healers Committee (THC). The CSIR entered into a similar MoU with the South African San Council during March 2002 and signed a BSA in March 2003. The decision of these owners of IK to provide their knowledge exclusively to CSIR Biosciences was based on its ability to manage confidential information and to add value through development of scientifically validated products. This work includes the development of a specialised database to capture and safeguard indigenous knowledge on medicinal plant remedies.

Scientific validation of traditional medicines

The CSIR’s bioprospecting research group has captured more than 250 claims for cures based on medicinal plants and completed desktop and literature studies on at least 50% of these to determine what research is already in the public domain, establishing the therapeutic area and identifying possible biological assays.

At least 80 claims for cures were identified for which the therapeutic concepts were established for different diseases, e.g. asthma, arthritis, malaria and HIV. Samples for at least 33 claims were tested for efficacy in suitable biological assays of which 16 demonstrated positive results and are further being developed (Figure 2). The scientific evidence of the efficacy of these traditional claims provided data for the process required for further development into validated herbal treatments or prescription drugs.
The 17 claims that did not give a positive result in the chosen biological assays cannot be ruled out as alternative biological assays need to be sought and the possibility of novel mechanisms of action investigated. This supports the holistic approach to testing of traditional medicines rather than a reductionist one. Currently, the 16 leads are in development for therapeutic areas including cancer, asthma and allergies, arthritis, inflammation, benign prostatic hyperplasia, malaria, HIV, TB, erectile dysfunction/libido, pain and diabetes.

Bioprospecting leads under development:

- BP4, 19 and 24 for treatment of asthma and allergies
- BP5 for treatment of benign prostatic hyperplasia
- BP16 for treatment of arthritis
- BP25 and 43 for pain
- BP21 for treatment of erectile dysfunction and improving libido
- BP23, 29 and 36 for treatment of HIV
- Ref 25 and 31 for malaria
- BP46 for tuberculosis
- Ref 31 for cancer
- BP47 diabetes.

One of the leads currently being developed and described in more detail, is BP4, a novel herbal extract for the management of asthma and allergies.

**BP4: treatment of asthma and allergies**

The traditional use of the plant *Siphonochilus aethiopicus* is for the treatment of mild asthma, colds, influenza and sinus problems [4]. Literature studies on the specific plant provided anecdotal information but little scientifically evaluated biological data.

Freshly harvested rhizomes were washed, sliced, dried and ground under GMP conditions in the CSIR’s botanical supplies unit, to give raw material suited for further processing. The following processes were investigated:

- Extraction with diethyl ether and ethanol
- Steam distillation to give an essential oil
- Chromatography of both the ether extract and the essential oil to give a pure sesquiterpenoid compound as the active ingredient.
- The sesquiterpenoid also spontaneously crystallised on the aqueous phase of the steam distillation condensate.

Processed material derived from the plant were biologically assayed in various enzyme-based assays involved in asthma and allergies. Significant activity was observed for the diethyl ether extract in the phosphodiesterase 4 enzyme assay (57% inhibition), the glucocorticoid receptor binding assay (77% inhibition) and the 5-lipoxygenase enzyme assay (101%) at a 100 μg/ml extract concentration. Dose response studies were also completed for the extract against the three targets. The extract was most active in the glucocorticoid binding site with an IC50 of 12.9 μg/ml indicating that the plant has a similar action as corticosteroids for the treatment of asthma and allergies. The non-steroidal sesquiterpene (1) isolated as a major compound from the ether extract and the plant gave an IC50 of 11.4 μg/ml in the glucocorticoid binding site providing evidence that the compound is, in fact, responsible for the biological activity of the extract.

Figure 2. Progress in validation of claims for cures based on traditional medicines
From the outcome of the in vitro assays, a suitable in vivo animal model was selected to determine the anti-asthmatic and anti-allergic activity of the diethyl ether extract of the plant. Assays were performed at the University of Cape Town and reference standards used as an integral part of each assay to ensure the validity of the results obtained. The study aim was to determine the efficacy of the diethyl ether and the ethanol extract against asthma/allergy in vivo by comparing mice treated with the extracts with untreated mice and mice treated with a standard corticosteroid drug, dexamethasone. An illustration of the dosage regime is shown in Figure 3.

![Dosage regime illustration](image)

Figure 3: Dosage regime

The extracts were given at a dose of 500 mg/kg through intra peritoneal injection (i.p.) twice daily for three consecutive days and an additional final dose was added at one hour before challenge of melachonin on testing day. The results showed that the dexamethasone group had less cellular infiltration and mucus production in the lungs and decreased numbers of eosinophils and neutrophils (Figure 4) in the bronchial lavage fluid compared to the no drug control. A similar effect was seen for the eosinophils and neutrophils by the diethyl ether extract, Figure 4, demonstrating the anti-inflammatory/anti-allergic property of the plant extract.

![Screening results for extracts](image)

Figure 4: In vivo screening results for extracts of *Siphonochilus aethiopicus*

Further studies are ongoing to determine the efficacy of the extracts in vivo through oral administration. The diethyl ether extract was chemically profiled using the HPLC MS. The method also quantifies the concentration of the active sesquiterpene compound (1) in the extract. The chromatographic profile is used as a quality control process to show batch-to-batch reproducibility. A PCT patent on the use of extracts and molecules derived from the plant was filed [5].

3. Conclusions

The focus of the bioprospecting platform has been on the development of prescription drugs and herbal remedies based on South Africa’s traditional medicinal plants as well as South Africa’s rich heritage of biodiversity. An insightful and strategic look at our results so far reveals a
very interesting and valuable data set that immediately provides scientific evidence demonstrating efficacy required for the validation of the claims of THPs.

The bioprospecting platform recognised the need to establish technology, currently lacking in South Africa, to evaluate and further develop calls from THPs with claims or anecdotes for herbal treatments. To date we achieved the following:

- Received and captured more than 250 claims for cures based on medicinal plants.
- Completed desktop/literature studies on at least 50% of these for the purpose of determining what prior research is already in the public domain, establishing the therapeutic area and identifying possible biological assays.
- Identified at least 80 claims for cures for which the therapeutic concepts were established for different diseases, e.g. asthma, arthritis, malaria and HIV.
- Tested samples for at least 33 claims for cures in suitable biological assays of which 16 demonstrated positive results and are in further development (referred to as bioprospecting leads). This has provided the first scientific evidence demonstrating efficacy of these traditional claims, and clearly providing data for the validation process required for further development into validated treatments or prescription drugs. While 17 claims did not give a positive signal in the chosen biological assays, they cannot be ruled out, as alternative biological assays need to be sought and the possibility of novel mechanisms of action investigated. This supports a holistic approach to testing of traditional medicines rather than a reductionist one.
- Currently, we have 16 leads in development for cancer, asthma and allergies, arthritis, inflammation, benign prostatic hyperplasia, malaria, HIV, erectile dysfunction, TB and diabetes.

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4. References


5. Acknowledgments

We thank CSIR Parliamentary Grant (PG) for funding and the THC for collaborative interactions.