# Chapter 10
# "Now I Heal with Pride"—The Application of Screens-to-Nature Technology to Indigenous Knowledge Systems Research in Botswana: Implications for Drug Discovery

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## Abbreviations

| | |
|---|---|
| CBD | Convention on Biological Diversity |
| IK | Indigenous knowledge |
| IKS | Indigenous knowledge systems |
| NME | New molecular entities |
| R&D | Research and development |
| STI | Sexually transmitted infections |
| STN | Screens-to-Nature |

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## 10.1 Introduction

The innovation potential of the pharmaceutical industry has come under considerable criticism. A recent analysis of research and development (R&D) and approval of new molecular entities (NMEs) including new biologics by the US Food and Drug Administration (FDA) between 1950 and 2008 revealed that the rate of production of new drugs had been stagnant [1]. The number of new drugs that are approved annually is not greater today than it was 50 years ago [1]. This is astonishing. Neither the exponential growth of investment in pharmaceutical research and development, nor the emergence of new biological technologies, nor the organizational restructuring of the pharmaceutical sector has significantly contributed to a higher rate of production of new drugs. Consequently, the study concluded that the innovative capacity of the established R&D model might have reached its limits, and the industry's efforts to embrace new approaches to innovation are of particular importance [1].

The chief of the European Medicines Agency (EMA), Thomas Lönngren, criticized the unsustainable R&D model of the drug industry by stating that of the estimated US $85 billion spent globally each year on R&D, around US $60 billion can be considered as wasted if one takes into account how few NMEs were produced [2]. One study, for example, reported that despite a 70% increase in drug research investment and related activities during the period of 1994 to 2004, there was an actual decline in launching NMEs [3]. One factor that limits successful approval is the high attrition rate during drug development. Only 1 out of 12 drugs entering clinical trials successfully emerges as a new drug. This is mainly due to the lack of appropriate bioavailability, poor pharmacokinetics, and significant adverse effects [2, 4].

The safety of new drug candidates is the defining issue in drug development, but it is usually addressed relatively late in the process. Rather, overemphasis has been on nonclinical aspects of drug discovery, and this has caused many drug candidates that have traveled far in the "pipeline" to fail drug approval [2]. "Never before did scientists have to consider (. . .) safety (. . .) aspects so early in R&D as they must today," admit research strategists in the pharmaceutical industry [5]. In line with this statement, the question is how can pharmaceutical research and classical drug discovery processes gain new dynamics of producing safer drug candidates in a less wasteful way?

One attractive strategy emerges from a new school of thinking in pharmaceutical research termed "reverse pharmacology." In this approach, documented clinical experiences are integrated with experimental whole-systems observations at the beginning of a drug discovery process. The purpose is to identify leads which are subsequently developed into drug candidates by detailed laboratory preclinical and clinical studies [6]. This approach reverses the classical practice of "laboratory to clinic" to a "clinic to laboratory" process [7]. That way, safety issues receive attention in the initial phases of the drug discovery processes and not at the end.

Subsequently, researchers have called for indigenous knowledge[1]-based platforms in combination with systems biology to guide drug discovery [8–11]. This approach considers the richness and diversity of natural product-based traditional medicines with its documented long-term human use as a strength. This is not only more effective to screen for bioactivities [12] and to (re)discover new molecular entities from natural products [13–16], but such an approach serves to address safety issues early on in the drug discovery process. Therefore, it is estimated that the drug discovery and development phase can be reduced significantly in both time and costs [6]. For instance, the reverse pharmacology approach used in the development of an antimalarial phytomedicine in Mali resulted in the introduction of a new effective standardized herbal antimalarial remedy after 6 years of research at a cost of only 400,000€ [17] compared to estimated costs of US $1–1.5 billion using the classical path of drug discovery [6].

However, efforts to develop indigenous knowledge-guided platforms for drug discovery bring in their own dynamics and challenges. Particularly, an inadequate intellectual property rights regime in relation to indigenous knowledge systems is an issue of major importance. Unevenly developed access and benefit-sharing mechanisms for indigenous knowledge holders and their communities have led to widening gaps between indigenous knowledge holders and researchers, culminating in mutual mistrust. Unethical practices of exploiting natural resources for commercial purposes by researchers and companies without the consent of and benefit for involved indigenous knowledge holders and their communities have contributed to an atmosphere in which it is difficult to establish collaborations.

Even though there have been attempts in recent years to protect indigenous knowledge holders through important international agreements, such as the Convention on Biological Diversity (CBD), there are challenges in implementing the legal frameworks under these initiatives. For example, a recent compilation of case studies on how the CBD provision on access and benefit sharing is implemented in relation to the exploration of biodiversity for commercially valuable genetic and biochemical resources ("Bioprospecting") revealed that

[1] We define indigenous knowledge in a broad sense referring to established knowledge originating from or adapted to a local context. Indigenous knowledge systems refer to harnessing of indigenous knowledge to produce a particular product or result. However, the term "indigenous knowledge" does not exist as such in Setswana and other local languages in Botswana. The term which is used by communities is "kitso ya setso," and traditional healers in Botswana call themselves "dingaka tsa setso." "Setso" implies a relation to history and culture and can be translated into "traditional" if one takes into account that history and culture are dynamic. Therefore, we use the term indigenous knowledge in a more general context and the term "traditional" in a more specific context. We use the term "traditional healer" not "traditional doctor" to recognize the diversity of types of healers, which include herbalists, faith healers, sangomas, bone setters, traditional birth attendants, and other healing professions.

benefit sharing remains largely unresolved [18]. The study noted that there is a tendency to use secondary sources, such as databases, as surrogates for indigenous knowledge and that there is a trend to declare indigenous knowledge systems in general as a public domain for which it is perceived that no benefit-sharing schemes need to be established [18].

Some ethnopharmacologists had to admit that technological advances in drug discovery have only marginally contributed to a beneficial development of indigenous knowledge systems that would result in meaningful returns to indigenous knowledge holders. Particularly, the lack of transparency in the research process, the lack of access of indigenous knowledge holders to research results, and the absence of a continuous dialogue between indigenous knowledge holders and scientists were criticized [19]. Furthermore, the fact that many countries have not yet established policies [20] and comprehensive implementation mechanisms to promote, protect, regulate, and develop indigenous knowledge systems tends to encourage the view that indigenous knowledge systems are part of an informal sector, which does not match the standard of institutionalized science. For example, biomedical practitioners often state that they are in favor of collaborations with traditional healers, but prefer collaboration to be on their terms, such as limited to referral of patients from healers to biomedical practitioners [21, 22]. Similarly, biomedical scientists often use an extractive approach when interacting with indigenous knowledge holders. In many cases, researchers obtain plant material and information on its medical uses from traditional healers, but never share research results with them. Indigenous knowledge holders—usually well-respected individuals in their communities—are reduced to anonymous "informants" or "respondents" in research publications and remain completely alienated from the research process [23]. Furthermore, in the health sector where indigenous knowledge systems (IKS) are particularly attractive for drug discovery, the often unclear legislative status of traditional healers contributes to an atmosphere where they hesitate to enter into collaborations which would reveal healing practices, which are not necessarily covered by the law.

Therefore, in this chapter, we describe how we developed a relatively simple set of field-suitable screening methods to detect bioactivities in plants, the Screens-to-Nature (STN) Technology, into a participatory tool to initiate and consolidate collaborations between researchers and traditional healers, and evaluate the potential of this approach for drug discovery from natural products. The first section of the chapter gives a brief overview of indigenous knowledge systems research in Botswana. The following sections explain and illustrate our approach using the STN system in two regions of Botswana, assess whether the STN technology is a suitable tool for drug discovery, evaluate whether the STN system is beneficial to traditional healers, illuminate interfaces and innovation processes as they became visible during the application of the STN technology, and conclude by outlining challenges and perspectives as experienced by our approach.

## 10.2 Indigenous Knowledge Research in Botswana

Indigenous knowledge systems are rich and diverse in Botswana. This is reflected in the Botswana National Vision 2016 document, which states that "while much can be borrowed from other countries we will need to look within our resources and culture to find the sources of innovation that will allow us to shape our own future."[2] A number of policies and corresponding strategic plans describe the various domains of indigenous knowledge and recognize their potential for sustainable economic development, such as biodiversity and natural resources management, pharmaceuticals, agriculture, breeding, and animal management. For example, the recent draft policy by the Ministry of Infrastructure, Science and Technology recommends the "infusion of indigenous knowledge in the national R&D agenda" and favors "partnerships (that) should bring about integration of the indigenous knowledge and modern science in order to deliver culturally acceptable solutions for local problems" [24].

However, comprehensive implementation strategies and monitoring/evaluation plans are not established to translate the commitment to the development of indigenous knowledge systems into tangible results. Interfaces between research and indigenous knowledge, which would be important for a focused, competitive R&D strategy, such as drug discovery from natural products using an indigenous knowledge-guided platform are not materializing to full potential. This is so because regulations to address access and benefit sharing of natural resources, suitable intellectual property regimes, and guidelines on setting up ethically sound collaborations are largely absent. There is currently no policy in place that clarifies the legal status, rights, and needs of traditional healers and traditional medicine in general. As a consequence, indigenous knowledge systems research in Botswana remains largely fragmented and uncoordinated.

As of now, the Department of Chemistry at the University of Botswana has a long-standing and strong record of natural product and phytochemistry research, which has accumulated an in-depth understanding of a broad range of natural product compound classes, their bioactivities, and their applications in diverse areas including catalysis, synthesis, medicine, and agriculture [25–29]. The same applies to the Botswana College of Agriculture that has established research groups on pharmacognosy and agroforestry with emphasis on medicinally important trees and food plants [30–32]. However, the establishment of concrete research partnerships with indigenous knowledge holders and communities remains subjected to individual initiatives that have never been comprehensively evaluated.

To address some of these gaps, the Center for Scientific Research, Indigenous Knowledge and Innovation (CesrIKi) was founded at the University of Botswana in

[2] Vision 2016 (1997) A long term vision for Botswana—towards prosperity for all. Government of Botswana, Gaborone, Botswana.

2006 and became operational in 2007. The main objectives of this interdisciplinary center are:

1. To contribute to national economic development and poverty alleviation by exploring Botswana's comparative advantage in natural resources and indigenous knowledge systems
2. To translate IKS in innovative processes to the benefit of communities
3. To develop IKS conscious scientists who will have reciprocal relationships with both rural communities and the formal sector
4. To support a paradigm shift in pedagogical approaches to ensure that the local communities endowed with natural resources will actively participate in applied and basic research initiatives for the present and future development of generations of scientists

A first project funded by the United Nations Development Program Global Environment Facility Unit (UNDP-GEF) to document IKS in relation to human health and food systems sought to explore appropriate modalities for interaction with indigenous knowledge holders and their communities and to develop a solid base for long-term and mutually beneficial relationships that are transparent and respectful. In this framework, which spanned the period of 2008 to 2010, we developed and applied the STN systems as a participatory tool.

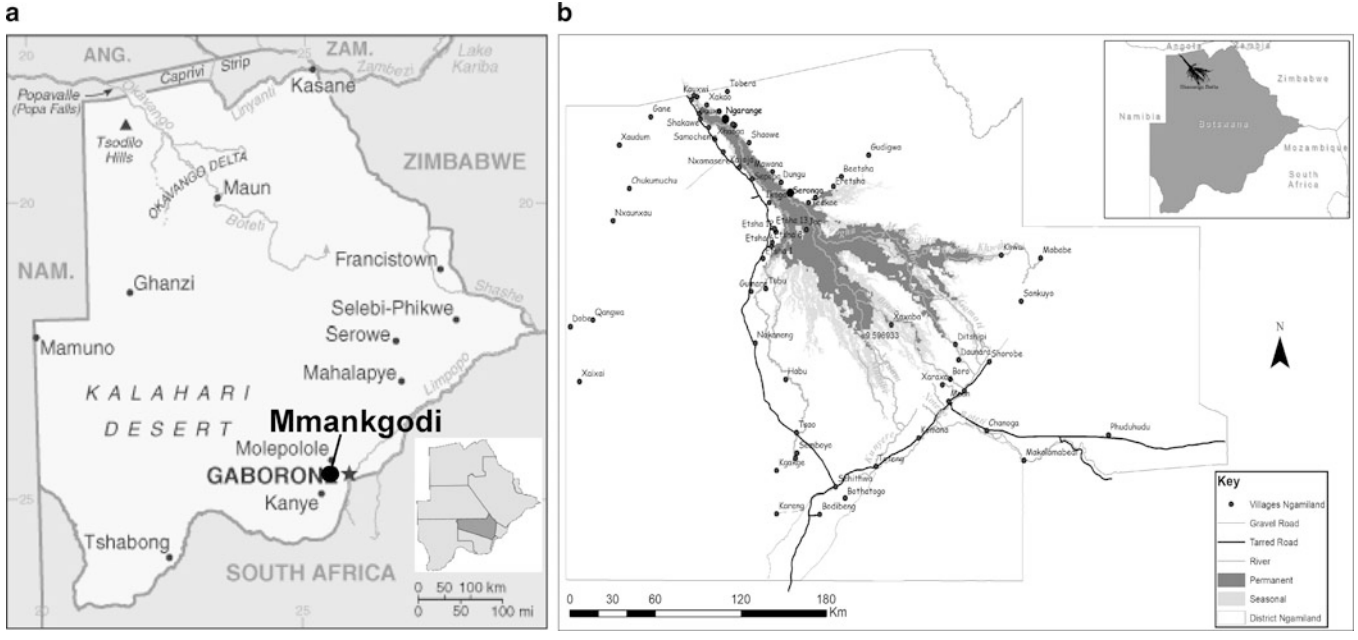
## 10.3 A New Approach: Participatory Research with the "Screens-to-Nature" (STN) System

The STN system is comprised of a set of field-suitable qualitative bioassays which detect various bioactivities of plant extracts. The screening system was developed through a collaboration between Rutgers University and the University of Illinois who jointly founded the Global Institute for Bioexploration (GIBEX) in 2003 with "a mission to empower scientists from the developing world to carry out their own therapeutic lead discovery and to promote sustainable exploration of local biodiversity for products related to human health" (for a more detailed account on GIBEX, see [33]). The STN bioassays focus on health targets which are relevant to the disease burden in developing countries, such as infectious agents (bacteria, fungi, protozoa), as well as enzymes and enzyme inhibitors associated with metabolic and infectious diseases (glucosidase-inhibitors, protease inhibitors) and on general health protection via antioxidant properties of phytochemical constituents [33, 34]. The STN bioassays are designed for screening of ethanol extracts which can be prepared in the field from 2 g plant material, and the assays use nonpathogenic model organisms for a first assessment of bioactivities. Some of the assays can be performed in a relatively short time and produce visible results within 10 to 30 min. This was attractive for us, as screening experiments could be easily accommodated within a workshop format.

We decided to approach communities and traditional healers from two different areas in Botswana. The first site was the village of Mmankgodi, situated approximately 35 km from the capital Gaborone in the Kweneng District (Map 10.1a). The Mmankgodi community consists of close to 5,000 inhabitants and unites the two major ethnic groups, the Bakwena and the Bahurutshe. This village was also chosen because it possesses an interesting blend of urban and rural influences. We first consulted the community through traditional customary structures called "dikgotla" which are headed by a local chief, the "kgosi," and subchiefs, "dikgosana." The project was introduced to representatives from each ward of the village, and ample space was given for expressing views and concerns. After obtaining permission from the "kgosi" and the community, one plant-testing workshop was conducted on May 30, 2008, with participation of the cultural cooperative of Bahurutshe Cultural Village in Mmankgodi. The cooperative was founded by women in the village mainly for the generation of small income out of the Cultural Village's activities, which attracts local and foreign tourists. Some of the women of the cultural cooperative are very knowledgeable in medicinal plants and were interested in small economic activities related to selling of herbs or simple herbal products. The second workshop was organized on March 18, 2010, with traditional healers from Mmankgodi. The women of the cultural cooperative contributed a selection of popular medicinal plants or their parts, and traditional healers collected together with us some plant samples on the hill where Bahurutshe Cultural Village is located. During plant collection, our botanist explained the importance of proper identification of plant species and illustrated ways of botanical preservation of the morphology of plants. Participants helped to grind the plant material and to prepare an ethanolic extract from selected medicinal plants.

These extracts were then used to perform three experiments in smaller groups detecting antioxidant activities, as well as protease and glucosidase inhibitory properties from plant extracts [34; see also Sect. 10.4.3]. Working in small groups allowed active participation in the experimental procedure (Fig. 10.1a, b) and gave ample space to explain the experiment, share the results, and discuss their implications and meaning (Fig. 10.1c). This approach is in striking contrast to previous experiences where scientists never gave feedback on laboratory test results. Our results were shared during the workshop and discussed. Examples included the fact that antioxidant activities might be useful in preventing strokes and that natural product-based protease and glycosidase inhibitors are potentially interesting in the area of drug development for diseases like HIV/AIDS, malaria, and diabetes. This created much excitement. Participants felt that they have actively learned something and, perhaps more importantly, felt that indeed their knowledge and "modern" scientific knowledge can work hand in hand resulting in new discoveries.

Encouraged by this positive experience, we conducted a workshop in a similar manner with traditional healers in Ngamiland District in Northwestern Botswana (Map 10.1b). Ngamiland District is the third largest in the country and the most ethnically diverse. The district possesses a unique ecosystem, the Okavango Delta, the largest inland freshwater ecosystem in the world. The oldest inhabitants of the




**Map 10.1** Study sites. (**a**) Location of the village of Mmankgodi in Kweneng District and (**b**) Ngamiland District. Note villages along the main road south and southeast of the Okavango Delta (Kareng, Bodibeng, Sehitwa, Toteng, Maun, Chanoga, Makalamabedi, and Shorobe)

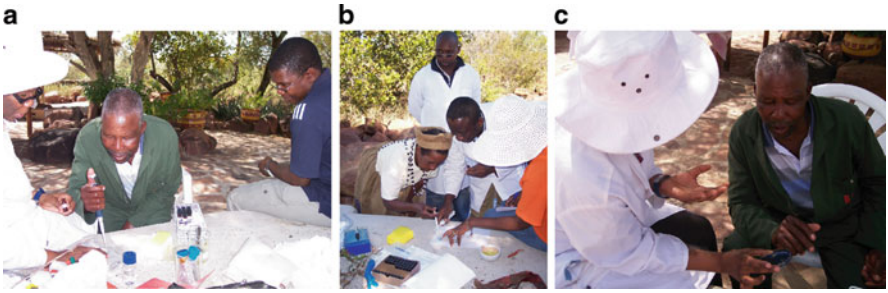



**Fig. 10.1** Participation in and discussion of STN assays. A traditional healer (**a**) and a woman of the Bahurutshe cultural cooperative (**b**) assist in a STN assay whose results are discussed (**c**)

Okavango basin are the Basarwa or San, a collective name used for several groups of Khoisan-speaking people. Ethnic groups in the western part of the Okavango are HamBukushu, Bayei, Bakgalakgadi, and BaSubiya. Over 95% of people in the Okavango Delta directly or indirectly depend on natural resources found in the wetland to sustain their livelihoods [35]. Many of the close to 3,000 plant species in Botswana are found in this area.

Fifty-five traditional healers and public health workers originating from eight different villages in Ngamiland, including Kareng, Bodibeng, Sehitwa, Toteng, Maun, Chanoga, Makalamabedi, and Shorobe, participated in the plant-testing workshop on September 23, 2008, in Maun, the capital of Ngamiland. Traditional healers brought their medicines and prepared extracts. Public health workers helped with pipetting. In the same manner as described above, three groups conducted three different experiments and shared results, which were discussed collectively after displaying them on posters. Again, traditional healers appreciated that researchers were not hiding results, but shared them and that researchers took time to explain. As this workshop included public health workers, mainly nurses and health education officers from local clinics, a new dynamic was noticeable. For the first time, public health workers could see that indeed traditionally used medicinal plant extracts do have effects, which can be scientifically detected. As the workshop concluded, traditional healers contributed 55 plant samples for further testing, for antibacterial, antiprotozoal, and other STN assays, which required more time and could not be performed during the workshop.

Our concept was to discard the notion that indigenous knowledge holders are reduced to "informants" and work towards an approach that sees traditional healers as true research partners, who have the right to transparency and access and benefit sharing. Accordingly, after having performed all STN assays available at that time, we organized a "report-back" workshop on the April 23, 2009, with 61 participants (Fig. 10.2). Recognizing that not all healers would feel comfortable discussing their therapeutics in public, healers received individual reports with test results for the plant samples they had submitted, and they were awarded certificates of appreciation for their contribution to IKS research in Botswana. Public health workers

**Fig. 10.2** Report-back workshop shares STN results with traditional healers and public health workers in Maun, Ngamiland District

received a summary report that showed percentages of plant samples displaying antibacterial, antifungal, antiprotozoal, enzymatic, and enzyme inhibitory activities. Then, a discussion that led into charting a way forward followed. Hence, follow-up activities and future collaboration on more specific questions were agreed upon.

Since then, CesrIKi has managed to extend and maintain a network of traditional healers who consistently submit plant samples for STN testing. This is also supported by the Botswana traditional healer umbrella organization "Baitseanape ba setso." At a general dissemination workshop that took place on October 7, 2010, the president of the organization, Mr. Setilo, publicly stated the full support of his organization for CesrIKi's efforts to build research partnerships with traditional healers.

## 10.4 STN: A Suitable Primary Screening Tool for Drug Discovery

How relevant are these primary screening assays for drug discovery processes? To answer this question, we summarize in this section some results we obtained during the 2-year STN program at the University of Botswana. We made use of the above-described established network of traditional healers and community members who were knowledgeable in medicinal plants and encouraged them to submit plant samples. We emphasized the importance of correct plant identification and asked healers to provide a sample for botanical authentication, or, alternately, STN team

members went with healers into the field and directly collected plant samples for proper botanical identification. Voucher specimens were deposited in the University of Botswana Herbarium.

### *10.4.1 Investigated Plant Species: A Snapshot of Botswana's Diversity*

In total to date, 621 plant samples have been investigated using the STN system. Close to half of the samples (47%) were provided by traditional healers. Sampling of approximately half of the remaining 53% was mainly guided by available literature on medicinal uses of plants and can be considered as indirect ethnobotanical sampling, while the rest of plant samples were collected randomly. The overall number of plant samples included samples that represent different plant parts of the same plant species, as well as "repetitions" of the same plant part from the same species, but collected at different times of the year and at different geographical locations. Of these, 515 plant samples (83%) have been botanically authenticated, while the identity of 106 plants (17%) remains unknown, though in most cases their vernacular name could be documented. The total number of botanically authenticated plant samples represents 214 different plant species of which 178 (83%) are indigenous to Botswana, 33 species (15%) are exotic species (based on classification according to [36]), and three plant species (2%) are of unknown provenance.

The plants investigated encompassed 71 of the 182 families found in Botswana [36] (Table 10.1). The most frequently investigated families were Fabaceae and Asteraceae, which contributed 33 and 20 different species and are known to be large and diverse families. Lamiaceae, Euphorbiaceae, Asphodelaceae, and Apocynaceae were also investigated with 10, 10, 9, and 8 species, respectively. Many of these families are well documented as possessing medicinal properties [32, 36–38].

A wide range of plant parts were investigated, including underground organs (roots, tubers, bulbs, and corms), flowers, fruits, shoots, stems and twigs, seeds/pods, and mixtures of two or more different plant parts. Most of the plant samples tested with the STN system were extracts from underground organs (47% of all samples), followed by leaf (27%) and bark extracts (4.8%) (Fig. 10.3). The prominence of underground organs of plants concurs with previous findings that the plant part which is mostly used in traditional medicines in Botswana is the root [23]. This differs from some reports about medicinal plant uses in other countries. In South America leaves principally contribute to traditional medicinal preparations [39], possibly explained by the differences in the countries' climates (dry versus tropical respectively).

**Table 10.1** Plant families of species screened by the STN system

| Plant family | No. of species | Plant family | No. of species |
|---|---|---|---|
| **Asteraceae** | **20**[a] | Malpighiaceae | 1 |
| Alliaceae | 1 | Malvaceae | 6 |
| Amaranthaceae | 5 | Meliaceae | 1 |
| Anacardiaceae | 7 | Moraceae | 1 |
| **Apocynaceae** | **8** | Moringaceae | 2 |
| Araceae | 1 | Myrothamnaceae | 1 |
| Asclepiadaceae | 1 | Myrtaceae | 1 |
| Asparagaceae | 6 | Ochnaceae | 1 |
| **Asphodelaceae** | **8** | Oleaceae | 2 |
| Bignoniaceae | 1 | Orchidaceae | 1 |
| Bombacaceae | 1 | Papaveraceae | 1 |
| Boraginaceae | 2 | Pedaliaceae | 4 |
| Burseraceae | 2 | Plumbaginaceae | 1 |
| Cannabaceae | 1 | Poaceae | 2 |
| Capparaceae | 7 | Polygalaceae | 1 |
| Celastraceae | 2 | Polygonaceae | 2 |
| Clusiaceae | 1 | Pteridaceae | 1 |
| Combretaceae | 5 | Rhamnaceae | 1 |
| Commelinaceae | 1 | Rubiaceae | 2 |
| Convulvulaceae | 3 | Rutaceae | 2 |
| Crassulaceae | 2 | Salvadoraceae | 1 |
| Cucurbitaceae | 4 | Sapindaceae | 2 |
| Cyperaceae | 2 | Selaginellaceae | 1 |
| Dracaenaceae | 1 | Solanaceae | 7 |
| Ebenaceae | 4 | Sterculiaceae | 2 |
| **Euphorbiaceae** | **9** | Thymelaeceae | 1 |
| **Fabaceae** | **33** | Tiliaceae | 2 |
| Flacourtiaceae | 1 | Verbenaceae | 5 |
| Hyacinthaceae | 3 | Vitaceae | 2 |
| Hypoxidaceae | 1 | Welwitschiaceae | 1 |
| **Lamiaceae** | **10** | Zygophyllaceae | 2 |
| Lauraceae | 1 | Unknown | 4 |

Total number of families: 71

[a]Plant families contributing the largest number of species are indicated in bold

### *10.4.2 The Screening Platform*

The high HIV/AIDS prevalence rate of 15–28% in Botswana [40] has reconfigured the pattern of antimicrobial infections in the country. Opportunistic infections, such as tuberculosis and sexually transmitted infections (STIs), as well as emerging multidrug (MDR) and extensively drug-resistant (XDR) mycobacterial strains and resistant causative agents for STIs, have become a major public health threat. Therefore, we modified the original STN set of bioassays by including pathogenic bacterial strains, which enabled us to obtain more specific screening data on

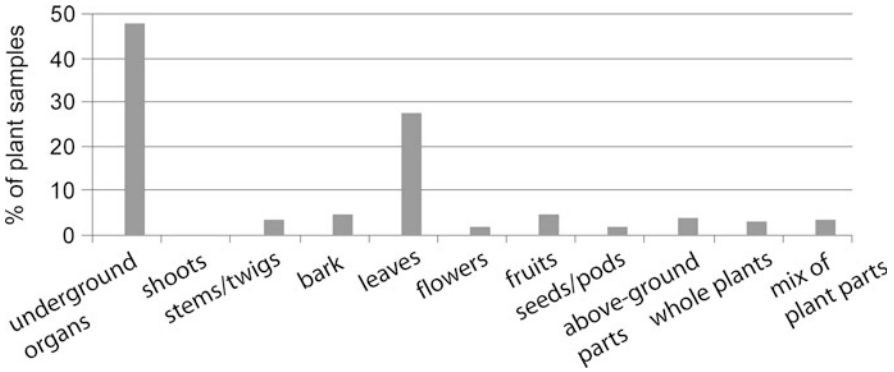



**Fig. 10.3** Plant parts investigated with the STN system

clinically relevant pathogens or pathogen model organisms. In addition to using nonpathogenic bacterial strains found in saliva as simple model organisms to detect general antibacterial activities when exposed to plant extracts [34], extracts were screened for inhibitory properties against *Mycobacterium aurum*. *M. aurum* is a fast-growing, nonpathogenic mycobacterium, which has very similar drug susceptibility characteristics as *Mycobacterium tuberculosis*. Because of the structural similarities of mycolates, which are responsible for the permeability of the cell envelope for antimicrobials, *M. aurum* has been recommended as the most suitable model organism to identify new potential therapeutics against *M. tuberculosis,* particularly cell wall inhibitors [41, 42]. Furthermore, we included *Neisseria gonorrhoeae*, the causative agent of gonorrhea, in the STN-screening program based on the observation that treatment and/or management of STIs is one of the major areas of expertise of traditional healers in Botswana [23]. Yeast infections, such as those caused by *Candida albicans*, constitute one of the major opportunistic infections in immune-compromised patients. Up to 90% of HIV-infected individuals suffer from at least one episode of candidiasis, which is commonly characterized by the development of oral thrush [43]. The STN system uses simple baker yeast (*Saccharomyces cerevisiae*) as a model organism to screen for antifungal bioactivities. The STN bioassay panel comprised further the detection of α-glucosidase and α-amylase inhibitors, which have attracted interest as potential therapeutic agents as antidiabetics [44] or as suppressors of the HIV replication cycle mainly at the entry stage [45], and trypsin protease inhibitors which have been implicated with anticarcinogenic [46] and anticoagulant activities [47].

Finally, two types of protozoa viability assays were performed using *Bodo caudatus* and *Acanthamoeba castellanii*. *B. caudatus* is a nonpathogenic member of the family Bodonidae, which is related to the genera *Leishmania* and *Trypanosoma* (Trypanosomatidae) by belonging to the same order of Kinetoplastida [48]. *Leishmania* and *Trypanosoma* include the pathogenic human parasites, such as

*T. cruzi* and *T. brucei*. The *B. caudatus* viability assay can therefore be considered as a preliminary screening system for anti-trypanosomal activity. Amoebae are a very diverse group of protozoans of which at least six forms are parasitic in humans. Most relevant of these is *Entamoeba histolytica*, which causes amebiasis and dysentery and remains a challenging health problem in developing countries with insufficient sanitation infrastructure [49]. *Acanthamoeba* is a genus common in soil and freshwater and is often found associated with water equipment, contact lenses, medicinal pools, ventilation, etc. *Acanthamoeba* have gained increasing clinical relevance mainly as causative agents of an often seriously progressing keratitis among contact lenses users [50] and are implicated in causing encephalitis particularly in an immune-compromised health context [51]. The STN system uses the nonpathogenic *A. castellanii* (Neff strain) to test anti-amoebic activity of plant extracts.

### *10.4.3 Methodology of STN-Screening Assays*

The screening of plant extracts using the above-mentioned organisms and enzymatic systems was performed as previously described [34]. Briefly, nonpathogenic bacterial strains from saliva, as well as *N. gonorrhoeae* and *M. aurum* ($OD_{550} = 0.5$, equivalent to $600 \times 10^6$ c.f.u./ml according to McFarland standard), were cultivated on LB agar, 7H8 agar supplemented with 10% Oleic/Albumin/Dextrose/Catalase (OADC), and Thayer-Martin agar, respectively, in 48-well plates in the presence or absence of ethanolic plant extracts and incubated overnight or for 48 h. The presence or absence of bacterial colonies was observed from duplicate assays classifying the complete absence of colonies as antibacterial activity. Penicillin, streptomycin, and rifampicin were used as controls.

The viability of *S. cerevisiae* and the protozoa *B. caudatus* and *A. castellanii* after exposure to ethanolic plant extracts for 24 h in 96-well plates was detected using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) as viability indicator. Econazole and $CuSO_4$ were used as controls for *S. cerevisiae* and protozoa, respectively. Antifungal and antiprotozoal activities of plant extracts were detected from duplicate assays by the lack of violet color formation from MTT (violet color indicates a metabolically intact organism).

α-Glucosidase (isolated from pea shoots) and α-amylase (present in saliva) inhibitory properties of plant extracts were qualitatively evaluated by exposing enzymes to solidified starch agar in the presence or absence of plant extracts. The intact starch surface was visualized with aqueous iodine-solution which results in a dark blue pigment formation.

Lastly, protease inhibitory plant extracts were identified using gelatin-coated X-ray films. The gelatin top layer on an X-ray film strip was exposed to trypsin alone as control or to trypsin in the presence of plant extracts.

**Table 10.2** Bioactivities determined by the Screens-to-Nature (STN) system

| Assay | No of samples tested | Frequency | |
|---|---|---|---|
| | | No of positives | % |
| Anti-bacterial | | | |
| General (Saliva) | 254 | 58 | 23 |
| *Mycobacterium aurum* | 373 | 42 | 11 |
| *Neisseria gonorrhoea* | 240 | 80 | 33 |
| Anti-fungal | | | |
| *Saccharomyces cerevisiae* | 592 | 95 | 16 |
| Glucosidase/amylase inhibition | | | |
| α-amylase (saliva) | 431 | 66 | 15 |
| α-glucosidase (pea shoots) | 467 | 41 | 9 |
| Trypsin inhibition | 599 | 199 | 33 |
| Anti-protozoa | | | |
| *Bodo caudatus* | 325 | 16 | 5 |
| *Acanthamoeba castellanii* | 128 | 10 | 8 |

### 10.4.4 The STN-Screening Results

In total, nine different STN bioassays were performed (Table 10.2). The most prominent bioactivities detected in plant extracts were anti-*N. gonorrhoeae* and trypsin inhibitory properties with 33% of all plant samples displaying positive results. In comparison, bioactivities of plant extracts inhibiting growth of *M. aurum* were detected with 11%, considerably lower than anti-*N. gonorrhoeae* activities, which can most likely be explained by the unique cell wall composition of mycobacteria which effectively prevents penetration of a wide range of compounds [52]. Alpha-glucosidase inhibitor and antiprotozoal assays appeared much more discriminatory, detecting α-glucosidase inhibitors in 9% of extracts tested, anti-*B. caudatus* activities in 5%, and anti-*A. castellanii* activities in 8% of all tested samples. The low percentage of antiprotozoal activities might reflect the fact that plants do have numerous antibacterial and antifungal defense mechanisms, but possess lesser secondary metabolites active against protozoa of this type. Inhibitory activities against bacterial strains from saliva, α-amylase inhibitors, and antifungal activities were detected in a similar range of 23%, 15%, and 16%, respectively.

### 10.4.5 Correlation of Bioactivities with Plant Families

Although the broad distribution of plant samples over a high number of plant families resulted in a relatively low sample size for evaluating a correlation between bioactivities and specific plant families, we observed some preliminary trends. For

example, a total of nine species of Euphorbiaceae have been screened, and in five or more species antimicrobial activities could be detected. In fact, 11% of species showing bioactivity in general antibacterial (saliva), anti-*S. cerevisiae*, and anti-*N. gonorrhoeae* assays were from Euphorbiaceae. This supports recent literature documenting the use of Euphorbiaceae plants against sexually transmitted diseases [37]. Species from the Fabaceae showed significant trypsin inhibitory activities. Out of 33 species investigated, 20 inhibited trypsin in the STN assay. Thus, 19% of the total plant species inhibiting trypsin (106 species) were from the Fabaceae. Extracts from the species of the Solanaceae showed remarkable α-glucosidase inhibitory properties. Five out of seven species inhibited α-glucosidase, attributing 17% of plant species with this bioactivity to the Solanaceae. All four species of the Verbenaceae displayed anti-yeast activity, but no significant other properties as detected by STN assays. This finding correlates with reports of anti-yeast activity (*C. albicans*) of certain species within this plant family [53].

### 10.4.6 Correlation of Bioactivities with Different Plant Parts

We then chose three examples of bioassays to evaluate whether bioactivities correlated with a specific plant part from which the extract was prepared. For example, in 43% of underground parts extracts and in 30% of leaf extracts, anti-*N. gonorrhoeae* activities could be detected, while the remaining 27% of positive bioactivities were distributed over a wide range of plant parts, including bark, flowers, stems/twigs, and above-ground parts. A similar distribution was seen when investigating anti-*M. aurum* activities. 52% of active extracts were prepared from underground organs, while 19% active extracts were obtained from leaves. α-Amylase inhibitors were found to be present in 52% of root/tuber extracts and in 32% of tested leaf extracts. These findings correlate reasonably with traditional medicinal uses attributed to specific plant parts where roots are mostly used in medicinal preparations, followed by leaves [23]. A slightly different picture was obtained when evaluating antiprotozoal activities. Underground organs dominated significantly where 80% of extracts displayed anti-*A. castellanii* and 69% anti-*B. caudatus* activities; no leaf extracts had anti-*A. castellanii* and 12% had anti-*B. caudatus* activities. A possible explanation is that the two types of protozoa are predominantly found in soil and groundwater and the root system would be the most suitable part of the plant to generate secondary metabolites as defense mechanism against parasites.

### 10.4.7 The Reliability of the STN Assays

All STN assays as described above generate qualitative results. In a first effort to establish whether the STN bioassays are suitable as a primary screening tool for drug discovery, we conducted quantitative follow-up assays using *C. albicans*, a

clinically relevant fungal organism, and *M. aurum* for rescreening the qualitative anti-*S. cerevisiae* and anti-*M. aurum* plant extracts. Based on the STN prescreening, a number of plant extracts with favorable minimal inhibitory concentration (MIC) and minimal fungicidal and bactericidal concentrations (MFC and MBC), respectively, could be identified [54]. Quantitative MTT viability assays confirmed 60% of detected anti-*B. caudatus* activities using the STN protozoa assay and 98% of anti-*N. gonorrhoeae* properties detected in plant samples screened by the STN system (unpublished results). We wish to note that we observed false-negative results, for example, when matching STN results for *S. cerevisiae* with low MICs obtained for anti-*C. albicans* plant extracts, as well as false-positive STN results when comparing with quantitative growth inhibition of *B. caudatus* mediated by plant extracts, or both when using *M. aurum* as screening organism for qualitative (STN) and quantitative viability assays. Furthermore, the reproducibility of STN results using plant samples of the same species and plant part varied. Antimicrobial assays showed a better reproducibility than enzymatic STN assays. One has, however, to keep in mind that the same plant species may vary in their secondary metabolite profile depending on different ecological parameters, such as soil and rainfall patterns, and on different seasons of the year. Therefore, the variability in reproducing qualitative results may not be a consequence of the unreliability of the STN system, but the tested plant material. After having established mutually trustful relations with traditional healers as described in Sect. 10.5, we are now able to collect plant samples *with* traditional healers and take—with their consent—GPS data of plant locations. More detailed studies to correlate bioactivities with natural plant habitats are therefore more feasible in order to comprehensively conclude the reliability of the STN assays. In conclusion, we found that the STN assays are useful as a prescreening tool to detect particularly antimicrobial activities from plant extracts.

## 10.5 Is the STN System Beneficial to Indigenous Knowledge Holders?

When we introduced the STN system as a participatory research tool earlier in this chapter (Sect. 10.3), we described the excitement of community members and traditional healers with participating in scientific experiments characterizing "their" medicinal plants and with sharing of the results. This, however, might only be the perception of the researchers. In order to evaluate how indigenous knowledge holders perceive the research partnership in the framework of the STN system, we found it more appropriate to ask them directly. Therefore, we conducted a survey soliciting the opinions of 28 traditional healers with whom we worked closely in the Ngamiland District.

The participants in this survey covered a wide range of different types of healers including herbalists and bone setters (herbalists who use a set of bones "ditaola" to

**Table 10.3** What interested you most about plant testing?

| | Frequency | Percentage (%) |
|---|---|---|
| Learn more about plant activities | 8 | 31 |
| Some form of validation | 8 | 31 |
| Understand plants to heal patients better | 4 | 15 |
| Cooperation with scientists | 4 | 15 |
| Learn about contents and toxicity | 1 | 4 |
| Conserve knowledge for further generations | 1 | 4 |

$N = 26$

**Table 10.4** What did you learn from plant testing

| | Frequency |
|---|---|
| Better understanding on how plants work | 10 |
| Plants are useful in medical treatments | 6 |
| Scientific validation is possible | 5 |
| Plants have value and should be preserved | 4 |
| Will better attend to patient confidentiality | 3 |

$N = 26$

communicate with ancestors) as the majority (9/28 each), herbalists who are also faith healers (6/28), faith healers only (2/28), and traditional birth attendants (2/28). 82% of healers were male and 18% were female, which reflects the general male dominance of the healing profession in Botswana [23, 55]. Most of the traditional healers (22/28, 81%) first learned that medicinal plants can be tested for bioactivities when they were approached by us. Only 2 of 28 healers were in contact with researchers before. When we asked the participants whether they found the STN plant-testing workshops useful, all of the healers ($N = 28$) answered in the affirmative. Table 10.3 summarizes their answers when we inquired in more detail what interested the healers most about plant testing. Approximately a third of healers (31%) wanted to learn more about plant activities, and another third (31%) were interested in some form of validation of their plant uses. Some healers (15%) related plant testing directly to a healing context, and equally 15% were mainly interested in promoting the collaboration with scientists. Learning about contents and toxicity, and the conservation of knowledge for future generations were issues of interest to two healers. We also inquired whether healer's expectations had been met when the plant-testing workshops were conducted, and we asked the participants what they had learned from performing the STN experiments. Healers mostly answered that they have a better understanding on how medicinal plants work, that plants are indeed useful in medical treatments, and that scientific validation is possible. Interestingly, four healers stated that plant testing had encouraged them to protect and conserve the plants which they are frequently using and expressed their desire to protect their intellectual property in relation to plant uses, as well as in relation to their general knowledge about plants and their medicinal applications (Table 10.4). When

**Table 10.5** In what way has the research partnership benefitted you as traditional healers?

| | Frequency |
|---|---|
| Encouraged to share knowledge and work together | 10 |
| Better understanding of plant activities and a form of validation | 6 |
| "Now, I heal with pride"/Work with confidence/Researchers protect us from being undermined | 5 |
| General acknowledgement/appreciation | 4 |
| General increase of knowledge | 3 |
| More communication and publicity | 2 |
| Preservation of traditional knowledge | 1 |

$N = 26$

asked ($N = 26$) whether the plant testing has motivated healers to learn more about "their" plants, all of them stated that it had indeed increased their motivation to understand medicinal plants better.

In enquiring how traditional healers perceive their role in the research partnership with scientists, the majority of all respondents (11/28, 42%) described their part in the research partnership as being the provider of traditional medical knowledge and of medicinal plants. However, over a third of healers (9/28, 35%) emphasized that their role is mainly to contribute to collaboration efforts. One healer summarized, "Collaboration is important for us to be respected, not hidden" (T.G., 23.4.2009). Several healers emphasized that collaborative efforts should be based on elements of equality:

> "We are both learning. We as traditional healers contribute what we know and you as scientists take it further" (M.T. 23.4.2009)
>
> "We are giving what we know and you are giving what you know" (G.C. 23.4.2009)

Three participants (12%) saw their role as generally important, and the remaining respondents felt that they were not yet able to describe their role as they had joined the network only recently.

We then wanted to know whether the partnership with scientists had actually benefitted traditional healers. 93% of respondents (26/28) were of the opinion that it had, while two healers did not yet comment on it, as they were still new in the partnership. Table 10.5 summarizes in what way traditional healers felt they have benefitted from the research partnership. Though healers initially expected to learn more about their plants (Table 10.3), they saw the main benefit in the way the research partnership encouraged the sharing of knowledge and working together. One healer explained:

> "The main benefit comes from combining ideas and knowledge from different people with different ideas to give our healing respect" (M.M., 23.4.2009)

Another participant put the benefit in much more concrete terms:

> "I benefit a lot. I can get information, I get a certificate of appreciation, my work might be published. Through that, people will appreciate that my plants have been tested and do have value." (L.T. 23.4.2009)

**Table 10.6** What should be improved in the research partnership on plant testing

| | Frequency |
|---|---|
| Better cooperation with medical doctors, nurses, government and amongst healer's associations themselves | 8 |
| More and more regular workshops | 5 |
| It is fine as it is | 4 |
| Simpler language/more explanation | 2 |
| Better logistics | 2 |
| More healers should be encouraged to submit plants | 1 |
| Plant testing should take place closer to healers | 1 |
| There should be more work on conservation issues | 1 |
| Consequent follow-up on suggestions | 1 |

$N = 25$

Having gained a better understanding of plant activities and having seen some form of scientific validation was the second most important benefit for healers. Significantly, several healers expressed the feeling that they now "heal with pride" and that researchers helped them "from being undermined." One healer characterized how the STN research partnership was perceived:

> "There is equality. You researchers do not belittle us or look down on us" (M.N., 23.04.2009)

To us, this is one of the most important achievements of the application of the STN system to indigenous knowledge research. It shows that scientists can play a positive role in building trustful collaborations, which is—as in any research partnership—a precondition for establishing successful research platforms. According to the healers, other benefits included a general acknowledgement of their healing profession through science, a general increase of knowledge, as well as more communication and publicity. The last statements do reflect an increased interaction between healers themselves, as they were brought together by workshops, and the fact that the workshops were covered by local print media and radio stations, as well as in the national television channel (BTV).

Consequently, all healers ($N = 28$) stated that they would like to continue with the research partnership. When asked what kind of research should be conducted in the near future, the majority of healers suggested that generally more plants should be tested (4/26), but also more specific plants which are used for specific conditions (13/26). Here, traditional healers recommended primarily medicinal plants they use according to their specific areas of expertise, such as the treatment of STIs and other conditions. Interestingly, a considerable number of healers (7/26) suggested more research on medicinal plants potentially useful for the treatment/management of HIV/AIDS which indicates a sincere concern of healers to combine all efforts in combating the pandemic. Other suggestions included more research on the conservation of medicinal plants, an issue which emerged particularly after individual reports with testing results were given to the healers.

Finally, we wanted to know what should be improved in future collaborations. The majority of healers pointed toward one limitation of the current collaborative efforts, namely, the lack of inclusion of biomedical doctors, government representatives, and

representatives of healer's associations themselves (Table 10.6). Healers found it important to initiate closer collaboration and dialogue with sectors that still have reservations about traditional healing. Healers emphasized that collaboration has to have continuity and requested more regular workshops together. Though there were many suggestions for improvement, such as using simpler language, better logistics, having plant-testing facilities nearer to healers, as well as follow-up on suggestions made and encouraging more healers to submit plants, a number of healers were also of the opinion that the current model of collaboration was suitable as it stands.

## 10.6 New Dynamics, Interfaces, and Innovation

As much as interdisciplinary research platforms give rise to issues emerging from various interfaces between disciplines and in interchange within the socioeconomic and legislative science context, it is important to understand that the STN-research partnership with indigenous knowledge holders has also triggered much more than an increased interest in scientific evaluation of medicinal plants.

"Now that I know that they have activities and indeed are useful, this partnership has motivated me to conserve my plants," summarized one healer (N.M., 23.4.2009) reflecting the concern about a sustainable use of natural resources, which was expressed by many of his colleagues.

The issue of cultivation of medicinal plants has been a subject of debate among healers in our workshops. There seems to be an agreement that some medicinal plants can be cultivated and that it would be desirable to do so, while others, particularly medicinal plants which are associated with "bad luck" or plants whose prescription and use in treatment requires close monitoring by the healer, should not.

Other healers pointed directly to the issue of intellectual property rights, which is a legitimate issue on the interface of natural resources, bioactivities, and drug discovery: "The plant testing has motivated me to preserve and protect my intellectual property" (T.P., 23.4.2009). It is important to note that the question of intellectual property rights was placed here in a positive context. This might have been a consequence of the fact that we respected intellectual property as well. Each healer obtained an individual report summarizing the screening results, and results were discussed confidentially. It remained up to the healer whether and with whom results are shared. Protection of rights emerged as an issue in the framework of a transparent research partnership of mutual trust, instead of being used as a justification to maintain secrecy and a general unwillingness to engage in dialogue. One healer explained:

> "This partnership has shown me that knowledge should not be kept to ourselves. We should try to teach others so that we can unite" (T.T., 23.4.2009)

We noted that the STN testing of plants led to new views on how scientific results can be incorporated into healing. Though we explained in report-back


**Fig. 10.4** Labeling and vouchering of plant samples by traditional healers. (**a**) Various medicinal plants and (**b**) plant press invented by P. Magakwa (traditional healer)

workshops the limits of in vitro bioactivity tests and emphasized that it would be appropriate not to change an established treatment regime, healers quickly recognized new potentials: "Plant testing has shown us that our plants might be useful for other conditions which we have not treated before" (N.M., 23.4.2009). Though this might be seen as the most controversial aspect of the application of the STN system, since this type of screening was intended first and foremost as a noninterventionist research tool, healer's conclusions nevertheless reflect the fact that knowledge never stands still. Indigenous knowledge systems were never static, but developed and adapted and continue to do so over time under the influence of many factors [56]. Traditional healers have always experimented with combining different medicinal plants. They have reinvented their healing expertise and have adapted to "new" diseases, such as tuberculosis and sexually transmitted infections during colonial times [57] and HIV/AIDS in this century. Scientific findings will therefore contribute to the dynamics of traditional healing. However, it would be important to investigate whether, and to what extent, the STN system has influenced certain established healing practices or has triggered new treatment regimes to ensure that no bodily harm is caused on the basis of qualitative STN in vitro screening results.

Lastly, during our interaction with indigenous knowledge holders in the framework of the STN program, we emphasized the need to properly identify plant species. Healers quickly understood how different names in different languages for the same plant or the same name for different plants can lead to mistakes in plant-testing experiments. Figure 10.4 illustrates how healers reacted to the challenge of correct botanical authentication and how they tried to assist researchers in that endeavor. Figure 10.4a shows how two healers in Maun started to properly label and arrange their medicinal plants in their consultation hut. Figure 10.4b displays an innovative method of vouchering invented by a traditional healer using

simple material, such as cardboard and wire, to preserve the morphology of plant samples for botanical identification. The plant name and its use are recorded on the cardboard. Both examples illustrate that contrary to widespread assumptions that traditional healers are somewhat disorganized and have difficulties in grasping systematic scientific approaches, they emerged as reliable research partners, who adapted quickly to new methods and transformed some of them according to their abilities and means.

## 10.7 Conclusion

The Screens-to-Nature (STN) approach described in this chapter showed that a cooperation based on mutual trust between scientists and indigenous knowledge holders is possible, even in a setting where legislative regulations on indigenous knowledge systems, on bioprospecting, and on access- and benefit-sharing mechanisms are largely absent.

The STN approach positively contributed to collaborative efforts in a number of ways. Firstly, sharing of results throughout the screening process of medicinal plants was in stark contrast to extractive research methods and equipped traditional healers to assess the usefulness of the research partnership. Secondly, plant testing as a participatory research approach served to demystify science in a way that traditional healers saw opportunities to gain knowledge instead of "protecting" their knowledge from science. Thirdly, the STN screening contributed to developing consciousness of traditional healers in other areas, such as the protection and conservation of natural resources and intellectual property rights. This is important, as informed decisions on just access and benefit-sharing systems and an adequate intellectual property rights regime can only be made if indigenous knowledge holders are actively participating in the research process.

The STN-screening platform has yielded results that are acceptable to both traditional healers and scientists. The STN system emerged as a useful prescreening method particularly with respect to the identification of antimicrobials from natural products and has created an important interface between IKS and drug discovery research. This interface has been perceived as of strategic importance for efforts to build a pan-African Natural Products Library (pANPL), which is described elsewhere in this book, and in whose framework the STN screening is combined with mechanism-based screening assays for antiparasitic drug discovery.

The success of the STN program in bridging the gap between indigenous knowledge holders and scientists assisted in prompting the Ministry of Infrastructure, Science and Technology to commission CesrIKi with the development of a national Indigenous Knowledge Policy, that is, to address adequate access and benefit mechanisms, and is combined with respective implementation and monitoring plans. Taken together, we have come a step further toward an indigenous knowledge-based research platform for drug discovery in Botswana.

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