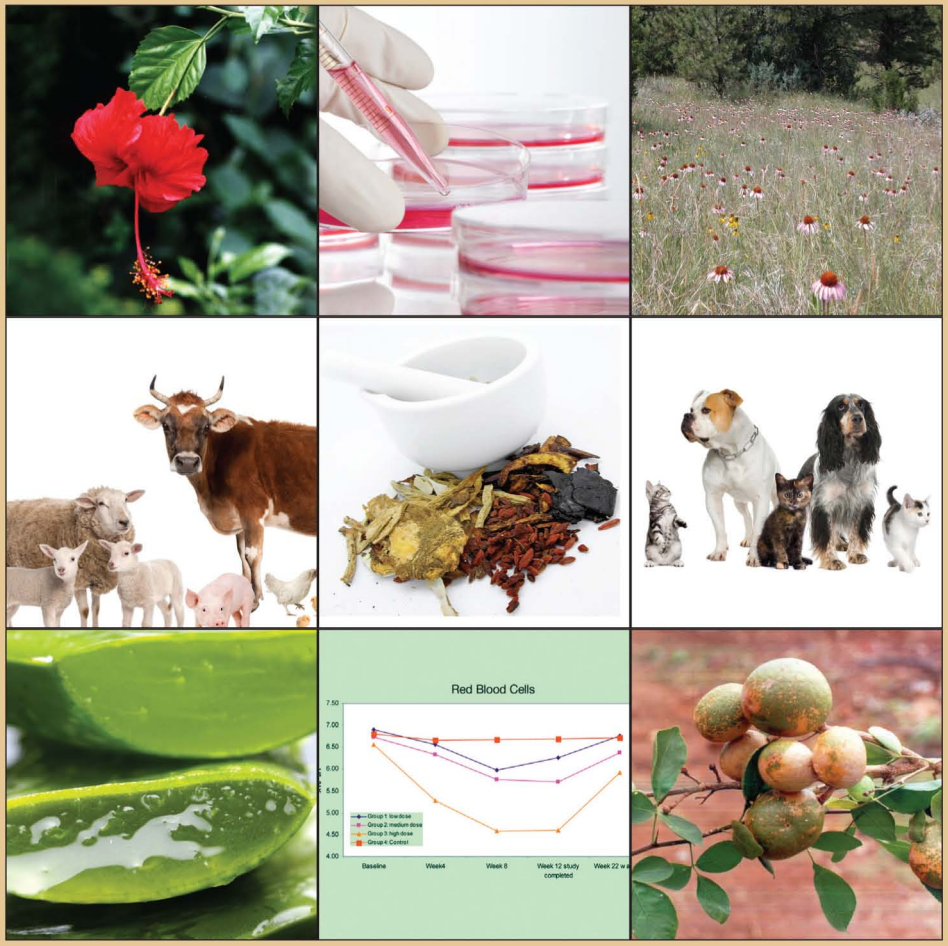
Ethnoveterinary Botanical Medicine

Herbal Medicines for Animal Health



Edited by David R. Katerere ▪ Dibungi Luseba

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Dedication

To those who went before us,
their knowledge makes us who we are,
and those who will tread after us
that they may keep and own it, too.

Contents

Foreword

From before written history, plants have been known as sources of medicines for treating human beings. In practically every human culture, there exists a vast treasury of information of this type, and in some civilizations, notably in China and India, this has developed into a sophisticated system of diagnosis, treatment, and preparation of the medicine.

As far as "Western" scientific medicine is concerned, natural substances were, and still are, the source of many isolated chemicals that are incorporated as the active constituents into familiar pharmaceutical dosage forms such as tablets, capsules, injections, and topical applications such as creams and ointments. The rise and application of pharmaceutical chemistry enlarged the potential of naturally occurring compounds for use as drugs since they could also be used as "lead molecules" to improve their efficacy or reduce toxicity by chemical manipulation of the structures. Traditional plant-based extracts became very marginal in most countries where "scientific" medicine became the norm.

However, in spite of the undoubted successes of such a scientific approach to pharmaceuticals, the last few decades have witnessed a spectacular rise in interest and use of "herbal medicinal products" (i.e., plant material or its crude extracts) in those places where sophisticated technologically advanced medicine was common, as well as in countries where it has long formed the mainstay of medicines used by ordinary people. This general interest has been followed by increasing scientific and commercial interest in traditional medicines, and in 1967 the term *ethnopharmacology* was coined to describe the scientific discipline investigating the use of these products.

Since that time, scientific investigations in this area have grown apace, as witnessed by the success of scientific journals such as *Journal of Ethnopharmacology* and *Phytotherapy Research,* as well as by good attendance at international conferences dealing with such matters and introduction of university courses. This has been fueled partly by the pharmaceutical industry seeking new lead compounds but also by an awakened interest and patrimonial pride in their traditions by many countries emerging from their colonial past. At one level, such research gives substance to traditional claims and reinforces the value of the cultural heritage, but if it demonstrates efficacy and safety, it might also lead to substitutes for expensive imported Western drugs. Since Western drugs are often beyond economic or geographical reach of many of the inhabitants of these places anyway, scientific study can also lay the basis for improving the quality of the traditional remedies, thus providing better grounds for efficacy and safety.

As far as the industrially developed countries are concerned, intensive farming has been widely practiced but has raised not only food production, but also many ethical and health concerns, giving rise to the "organic" preference of many consumers. Hence, the investigation of these traditional "natural" medicines might provide alternatives to current treatments of animals that have caused much concern, such as the widespread use of antibiotics in young animals, producing residues in food

and thereby the buildup of resistance in humans and other animals. The entry into the food chain of hormones and other steroids for building muscles and stimulating growth may affect human metabolism and health. The study of traditional medicines might provide compounds or extracts with novel structures or different mechanisms of action, with less-acute or chronic side effects, which would make good substitutes for the currently used drugs that are raising concern.

This interesting, and in some ways surprising, explosion of scientific interest and study into traditional materials for helping human health care has only fairly recently been enlarged to encompass use of traditional medicines for treating animals. Urbanized and relatively affluent members of society have a mainly emotional attachment to domestic "companion" animals, on which they are willing to spend considerable amounts of money, and it is not surprising that herbal products to treat domestic animals sell well. However, it should not be forgotten that, for most rural parts of the world, animals are important sources and symbols of wealth and livelihood. When the animal becomes sick or dies, it may mean the loss of transport, aid to farming, dairy products, meat, and other products such as wool or hair, which provide extra income or clothing. The death of a single animal may spell the beginning of poverty for a whole family.

It is therefore not surprising that in many societies there is a pharmacopoeia of substances used to treat livestock and poultry. The growing interest in this from scientists was reflected in a session dedicated to veterinary ethnopharmacology at the Congress of the International Society for Ethnopharmacology in São Paulo, Brazil, in September 2008. This book is another indication and is the first, to my knowledge, to bring together a considerable amount of information about ethnoveterinary medicines from a wide variety of countries. As well as listing plants used, the conditions for which they are used, and the ways that the plant material is treated prior to use, this book covers useful topics such as general research methods for testing claimed effects and safety and the chemical examination of extracts. The inclusion of a chapter on benefit sharing is particularly praiseworthy since it is all too easy for a culture to have its traditional knowledge "stolen" to be used commercially by an outside body that returns no benefits.

Dr. Katerere and Dr. Luseba have been fortunate enough to persuade leading authorities in this field to contribute chapters, and I am sure that it not only will prove to be a valuable reference source but also will stimulate further research into this fascinating area, with the end result of improving not only the health of animals and domestic birds, but also the well-being of their owners and farmers.

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We acknowledge the immense contribution that all the authors who are part of this book have made. Without them, this book would not have known the light of day, and we hope that this project will be the start of further collaborations among them in the future.

Last, we thank our families for the obvious sacrifices they have had to make during the duration of this project. Tinotenda.

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1 Methods for Evaluating Efficacy of Ethnoveterinary Medicinal Plants

Lyndy J. McGaw and Jacobus N. Eloff

CONTENTS

1.1 INTRODUCTION

In many developing countries with limited access to orthodox health care services, the majority of rural people rely on traditional medicines to alleviate a variety of ailments. Likewise, many pastoralists use customary remedies to treat their sick animals. Commercial pharmaceutical drugs are sometimes available in remote rural areas, but they are often dispensed by untrained vendors or repackaged without printed instructions, which may not be able to be read by illiterate users in any case, leading to drug misuse (Mathias, 2007). In an effort to improve animal health care services in rural areas, it is vital to utilize all available resources, including ethnoveterinary medicine (EVM).

The growing interest in and increasing recognition of the role of EVM has been limited in terms of further development by unavailability of information on the efficacy and safety of these practices. Several research papers in international publications have confirmed a heightened recognition of the need for rigorous scientific investigation of EVM remedies. Studies of biological activity of plants used against veterinary diseases can provide indications of promising leads for extracts that can be developed and used on a commercial basis. Plants with activity may also provide leads for isolation and identification of useful compounds that may be chemically modified to optimize medicinal value and reduce possible toxic effects, in other words, developed into pharmaceuticals.

For common conditions such as coughs, wounds, skin diseases, mild diarrhea, and reproductive disorders, EVM can be a cheap and easily obtainable alternative to expensive orthodox drugs. For epidemic infectious diseases, including anthrax, rinderpest, rabies, and foot-and-mouth disease, modern drugs (mostly vaccines) are preferred. Many drugs in conventional therapy are based on chemical compounds of plant origin or on synthetic derivatives of these chemicals. The search for alternative antibacterials and anthelmintics in particular is intensifying following problems associated with drug resistance and chemical residues in production animals. EVM practices, if proven to be effective and not harmful, may provide answers (not only regarding plant-based remedies but also concerning management customs) to some of these problems currently encountered in conventional veterinary practice.

Much research has been undertaken, especially as reflected by the expanding scientific literature, concerning the ethnopharmacological investigation of plants used to treat humans for various illnesses. This has been followed by an interest in plants used in animal health. It is logical that in evaluating these plants for biological efficacy and safety that similar bioassays are used in a laboratory situation where plants are being investigated for similar types of activity. It is essential to build and maintain standards for assessing the therapeutic potential of plants to facilitate comparison of results between different research groups (Cowan, 1999). In this chapter, methods for evaluating traditional ethnoveterinary plant-based remedies used for treating common diseases are discussed. Some results obtained in these assays with particular reference to the South African context are supplied. Considerations to be taken into account when embarking on research in this field are also presented and discussed.

1.1.1 The Need for Evaluating Traditional Animal Treatments

There is a pressing case for investigating EVM remedies for two major reasons: investigation of their use as effective agents for treatment of particular ailments and to appraise their safety. Undoubtedly, there are dubious, questionable, or even harmful practices forming part of alternative treatment of livestock, and these need to be distinguished from those of value. The profile of EVM would benefit from validation of certain practices in the view of potentially skeptical, conventionally trained veterinarians who may have only been exposed to the results of injurious handling by rural farmers attempting to rid their animals of disease or parasite infestations.

Other advantages to be gleaned from ethnoveterinary research entail prospects for commercial development, whether as refined extracts or lead compounds for the pharmaceutical industry. An important aspect, which falls outside the scope of this chapter, is the necessity for benefit-sharing agreements with owners of the intellectual property concerning EVM (see Chapter 2). This may involve returning value-added knowledge to the community where the information originated if such a community can be identified. Alternatively, they could be included in a profit-sharing arrangement from the outset, without raising initial false hopes of massive profits to be obtained from a product that may display exciting bioactivity in the laboratory, while many obstacles lie in the path to commercial success.

Various approaches have been proposed to validate animal herbal remedies, such as literature reviews, laboratory and clinical studies developed in medicine, social science methods (i.e., ranking of treatments in terms of efficacy by livestock owners), and investigation of the influence of a remedy on animal production and economic considerations (Mathias, 2004). Much work remains to be done in this area. In a review of the current status of published information on the ethnoveterinary use of plants in South Africa and biological activity and toxicity investigations on these plants (McGaw and Eloff, 2008), more than 200 plant species were documented as used in EVM, and of these, a mere 27 species have been tested for bioactivity in targeted ethnoveterinary assays. It was concluded that more plants need to be evaluated, and expanded investigation of those plants already tested in one or two screening systems needs to be carried out (McGaw and Eloff, 2008).

The targeted investigation of plants for commercial application in human medicine has resulted in natural products and their derivatives representing about 50% of all drugs in clinical use, of which higher plants contribute 25% to this figure (Farnsworth, 1984; O'Neill and Lewis, 1993). Plants have a seemingly limitless ability to manufacture unusual and original chemical structures. Also, a major mechanism plants possess to fight infection is the production of chemicals with anti-infective activity (phytoalexins); thus, it is logical and reasonable to explore the potential presence and ability of such compounds to be useful in both human and animal health care, for example, in terms of antibacterial or antifungal activity.

Ethnoveterinary remedies need to be validated before they can be widely promoted. Information on the botany and phytochemistry of particular plants may already be available in the literature, relating to bioactivity and toxicity. There is frequently an overlap between medicinal plants used to treat animals and those used to treat humans. It could be speculated that livestock keepers have over the centuries modified human remedies for use in animals or vice versa. It would make sense that similar treatments are used to treat comparable ailments in humans and their livestock.

Ethnoveterinary medicines can function as leads for drug development, but perhaps a more useful and cost-effective exercise would be to improve a selected preparation by pharmacological research and development, and the resulting remedy can be returned to the community with addition of value. Local farmers can grow the plants and make money from this venture, and such commercialization can also aid in the conservation of useful species. Other issues falling outside the ambit of this chapter include the necessity to conduct research into optimal dosing regimens and

effective concentrations of herbal remedies. Potential side effects as well as toxins that may be ingested by the animals and then transferred to humans through milk or meat are further issues to be addressed.

1.2 BIOLOGICAL ACTIVITY SCREENING

It has been estimated that at least 250,000 species of plants inhabit our world (Borris, 1996). A mere 5–15% of higher plants have been systematically investigated for the presence of bioactive compounds, so plant biodiversity is virtually unexplored (Pieters and Vlietinck, 2005). Plants contain an enormous diversity of chemical structures, which are secondary metabolites modulating the relationship of organisms with the environment, for example, as pollinator attractants, signal products, and defensive substances against parasites, predators, and other pathogens (Pieters and Vlietinck, 2005). Such compounds in plants therefore hold much potential for medicinal applications. Following up ethnomedical leads, whether plants are used in human or in animal medicine, is one approach to selecting plants for bioactivity screening. Other methods of bioprospecting for screening studies include random selection and chemotaxonomic selection approaches.

Bioassays used to evaluate plant extracts should meet many criteria, including validity, lack of ambiguity, accuracy, reproducibility, simplicity, and reasonable cost (Pieters and Vlietinck, 2005). More particular considerations are a high selectivity (to limit the number of leads for subsequent evaluation), a high sensitivity (to detect low concentrations of active compounds), and a high specificity (to be insensitive to a variety of inactive compounds and eliminate false positives) (Pieters and Vlietinck, 2005). *In vitro* tests in ethnopharmacological studies are prevalent in the scientific literature even though *in vivo* models supply more accurate evidence for the activity of plant preparations in traditional medicine. This is largely as a result of the fact that the use of *in vivo* models is severely restricted in many countries owing to economic and ethical concerns (Houghton et al., 2007). The smaller amount of plant material needed for an *in vitro* test is often an important consideration.

Methods of extraction and *in vitro* testing need to be standardized so that the evaluation of medicinal plants can be systematic, and comparisons of results obtained by different laboratories may be more useful. In an important review article on the subject of anti-infective potential of natural products, Cos et al. (2006) emphasized that certain pivotal quality standards must be set at the stage of extract processing and primary evaluation in pharmacological screening models. The authors provided extremely useful recommendations to help define a more acceptable "proof of concept" for antibacterial, antifungal, and antiparasitic activity in natural products. They outlined the following requirements for anti-infective screening:

1. Use of reference strains or fully characterized clinical isolates (in the case of microorganisms, the American Type Culture Collection [ATCC] strains are widely used as standards).
2. *In vitro* models on the whole organism, if possible cell based.
3. Selectivity evaluation by parallel cytotoxicity testing or integrated profiling against unrelated microorganisms.

4. Adequate dose range, enabling dose-response curves.
5. Stringent end-point criteria with IC_{50} values generally below 100 µg/mL for extracts and below 25 µM for pure compounds.
6. Proper preparation, storage, and in-test processing of extracts.
7. Inclusion of appropriate controls in each *in vitro* test replicate (negative, positive, and growth controls).
8. Follow-up of *in vitro* activity ("hit" status) in corresponding animal models ("lead" status).

A variety of test systems should be employed in the *in vitro* screens because the use of only one bioassay yields an incomplete picture of the effect of the extract on the whole system involved (Houghton et al., 2007). Many diseases involve more than one factor, and Houghton et al. (2007) cautioned that the use of a single *in vitro* test is generally too simplistic and reductionist to achieve an idea of biological activity. An assortment of chemicals is present in an extract, and these may each have a different biological or pharmacological activity, together contributing to the overall clinical effect (Houghton et al., 2007). It is therefore preferable to use a range of tests for different activities, all related to the particular disease state under investigation.

Pharmacological evaluation of medicinal plants followed by bioassay-guided fractionation can lead to the isolation of pure active compounds with potential for commercialization. An alternative to this route, particularly pertinent to developing countries, is the preparation of standardized, formulated extracts that could contribute to an innovative and successful local pharmaceutical industry (Pieters and Vlietinck, 2005).

A growing number of publications document the use of herbal remedies by small-scale farmers to treat an assortment of livestock diseases, from skin conditions to babesiosis and anaplasmosis (Masika, Sonandi, and Van Averbeke, 1997; Masika, Van Averbeke, and Sonandi, 2000; Dold and Cocks, 2001; Van der Merwe, Swan, and Botha, 2001; Njoroge and Bussmann, 2006; Luseba and Van der Merwe, 2006). Methods of obtaining information range from participatory methods to semistructured interviews, field observations, and questionnaire surveys. This expanding documentation of plants used in EVM is anticipated to precede increased investigation of these plants for bioactivity, reflecting the situation in human ethnomedicine. It is generally accepted that a great deal of work remains to be done on recording the uses of plants in EVM.

Factors to be taken into consideration by researchers aiming to evaluate the biological activity of herbal EVM remedies include whether the plants are used singly or in combination with other plants, the plant part used, method of preparation, dosage, and the way in which the remedy is applied. For example, herbal remedies can be prepared from fresh or dry material in the form of infusions, decoctions, pastes, or expressed juices from fresh plants. Masika, Van Averbeke, and Sonandi (2000) stated that the route and method of application of a remedy depends on the perceived cause of the disease condition. Topical applications are commonly used for skin conditions, powders are rubbed into incisions, drops are placed in the ears and eyes, and drenches are popular in treating systemic conditions. In other studies in South Africa, it was noted that plants are generally not processed or mixed with other materials and

are used as single-plant decoctions or infusions for dosing animals or crushed and used topically for wound treatment (Van der Merwe, Swan, and Botha, 2001; Luseba and Van der Merwe, 2006). This is in contrast with traditional medicine intended for human use, for which processing (milling, extracting, etc.) and mixing of two or more plant species (and even animal parts) appears to be common practice.

1.2.1 Limitations of Laboratory Testing of EVM Remedies

As EVM is a complex system of practices involving more than just the application of plant-based remedies to sick animals, it may give rise to misleading expectations about the degree of efficacy of a single plant used as part of a cure. Diagnosis of a disease made by a rural farmer may be inadequate as it is easier to identify symptoms than the actual cause of the disease. Dosages are not precise, contributing to the perception that the remedies are not standardized. The methodology for validating EVM should be scientifically acceptable but also must take into account the probability that EVM remedies might not work as powerfully as orthodox medicines. They may not completely eliminate all microorganisms causing a particular disease, but this could allow the body's immune system to build up immunity against the remaining organisms. It must be kept in mind that ethnoveterinary practices comprise a complex system and isolating one aspect for efficacy studies may not yield the anticipated results, although the system might be adequate for the conditions in the field. Fundamentally, if a plant-based ethnoveterinary remedy is to be deemed suitable for further development, efficacy and toxicity tests must meet certain standards. Legal constraints must be kept in mind if commercial development is anticipated. However, this standard is not so rigorous if the validation and understanding of EVM is the aim of the study. Research can be used to select a particular remedy, improve it through pharmacological and toxicological research, and then return it as a value-added product to the community.

In EVM studies, traditional methods of dosage and preparation of remedies are vital components often neglected for the sake of ease of preparation and standardization of laboratory extracts for testing. For a more true reflection of efficacy of the treatment, it would be advisable to closely follow if possible the preparation and application technique employed by the indigenous user. On the other hand, there is a case to be made for using standard scientific methods to prepare aqueous and organic solvent extracts if extraction of a wide range of chemicals present in the plant is desired for *in vitro* studies.

Traditional remedies may be composed of single plants or mixtures of plants, and if mixtures are employed it is recommended to test not only the individual component plants, but also the mixture in the correct proportions used traditionally. Repeatability of activity is important, and together with well-designed screening programs to elucidate activity using complementary techniques, plant material sourced from different areas could be included in a screening program. The accurate identification of plant material is naturally essential, and numbered voucher specimens should be deposited at a reputable herbarium to allow other researchers to verify the identity of the plants as well as to make allowances for possible future taxonomic revisions.

A further limitation in the laboratory testing of EVM remedies is the difficulty in culturing parasitic nematodes and protozoa, for example. Parasitic infestations of intestinal worms are commonly diagnosed and treated by livestock keepers. Some model systems are available, such as the free-living nematode *Caenorhabditis elegans*, for detecting anthelmintic activity, but sometimes the only true indication of efficacy is activity in an animal model.

It is often not possible to assume efficacy *in vivo* after achieving good results with *in vitro* tests. First, it may not be practical to extrapolate the dose from that which is active *in vitro* to that which would be required to reach adequate plasma concentrations in the target species (Houghton et al., 2007). Bioavailability is an essential consideration if the remedy is orally administered. Second, factors such as absorption and metabolism may be responsible for discrepancies between *in vitro* and *in vivo* tests (Houghton et al., 2007). Even if rat models, for example, are used, differences have been noted between intraperitoneal and oral administration. This can be explained inter alia by metabolic breakdown of compounds in the gastrointestinal tract or by lack of absorption from the gut into the bloodstream (Laupattarakasem et al., 2003). Absorption and metabolism can be affected by other compounds in the extract that may enhance or inhibit absorption, and other compounds may upregulate metabolic enzymes in the liver. Houghton et al. (2007) noted that traditional methods of preparation of plant-based medicines might remove or concentrate such compounds, and if the correct method is not followed in making extracts for pharmacological testing, then the extract may display different activities compared to the extract prepared according to traditional methods.

Most activity investigations in the published scientific literature concentrate on *in vitro* studies for practical, economic, and ethical reasons. Therefore, there exists an unavoidable bias toward *in vitro* tests for evaluation of EVM remedies as this is what is reflected in the available literature. Even in the case of *in vivo* studies, tests of ethnoveterinary remedies in a laboratory where the animals are given fixed diets and kept under controlled conditions, accurate indications of efficacy of the treatment may not be discovered, while in the real-life situation, conditions under which the animals are kept are more varied.

1.2.2 Extract Preparation

The variety of methods by which EVMs are prepared and administered to animals complicates the task of evaluating activity of a particular remedy. The reason for which the screening is being undertaken must be kept in mind when designing the study, including preparation of the extract for pharmacological testing. If the purpose is solely to validate the use of a certain remedy, then it is advisable to closely follow the traditional method of preparation as many factors may influence the activity of the resultant mixture. In addition, the route of administration must somehow be taken into account. In some cases, it is thus possible that *in vivo* tests are the only mechanism by which the efficacy of an EVM medicine can be verified. Alternatively, careful design of *in vitro* screening systems may yield a reasonable idea of the efficacy and nontoxicity of remedies. The selection of a screening system will for the most part depend on the nature of the disease being investigated

and the availability of validated laboratory models to identify the relevant biological activity.

For broader screening programs aimed at discovering biological activity in particular plants used in EVM, standardized methods are widely used. Care must be taken to ensure that potentially active compounds are not lost during processing; for example, some constituents may be thermolabile or photosensitive. Plant extracts may be prepared using fresh material or, more commonly, dried powdered material. The plant material can be extracted using water or organic solvents that vary in polarity. For extraction of hydrophilic compounds, polar solvents such as methanol, ethanol, and ethyl acetate can be used, while if lipophilic compounds are being targeted, more nonpolar solvents such as dichloromethane and hexane may be used. Eloff (1998b) examined a spectrum of solvents for their ability to extract antimicrobial compounds from plant material and other factors, including their hazardous nature and ease of removal from the extract. The aim of the study was to identify a more standardized extraction method, and acetone was highlighted as the solvent with the best rating, followed in order by dichloromethane, methanol, ethanol, and water. However, this may vary with the plant species or plant part under investigation (see also Chapter 4).

Following extraction, appropriate handling of the extracts is important to avoid decomposition of active compounds or other changes that may affect biological activity. It is common practice to resuspend dried extract residues in the extracting solvent to a known concentration prior to screening, provided the extract redissolves adequately in the solvent and the solvent is not toxic in the testing system. In quantifying the extract, some researchers dry down only a small aliquot of the extract to determine the original concentration and then use the remaining intact extract for testing. Dimethyl sulfoxide (DMSO) is a popular solvent in which to prepare test compound solutions at a stock concentration (Cos et al., 2006). Test stock solutions in 100% DMSO have the advantages of elimination of microbial contamination, thus obviating the need for filter sterilization, which may lead to loss of compounds, and good compatibility with many test systems as a result of good solubility when diluted to a working concentration in aqueous medium. As DMSO is potentially toxic for cells in tissue culture or microorganisms, the final testing concentration of the solvent should not exceed 1% (Cos et al., 2006). Acetone was put forward as the solvent of choice for use in antibacterial testing systems as, at the concentrations used in a serial broth microdilution assay, it was found to be nontoxic to various species of bacteria tested (Eloff, 1998a). This was also held to be the case for antifungal assays based on a similar method (Masoko, Picard, and Eloff, 2005). Regarding storage issues, Cos et al. (2006) recommended that compounds and extracts should generally be stored without solvent for long-term storage or in 100% DMSO at −20°C with minimal exposure to freeze-thaw cycles or humidity. This is meant to reduce degradation of components. Storage in methylated solvents is not advised because of the possible formation of artifacts.

In the following sections of this chapter, examples of EVM plants that have been screened for biological activity, the methods used to screen them, and indications of activity discovered in the plants are given. Particular emphasis is placed on treatments for those diseases of importance in livestock. Techniques available for bioassaying

the plant preparations are described briefly and references given for more detailed information. It is commonly found that there is an overlap between veterinary and human medicine in many communities, but the emphasis here is on the former.

1.2.3 Antibacterial and Antifungal

Different classes of antibacterial assays have been described, and many of these are applicable to antifungal detection as well. Antibacterial assays may be broadly divided into agar diffusion, dilution, and bioautography methods. In agar diffusion, a reservoir containing a known concentration of the test substance is brought into contact with an inoculated medium, and the diameter of the inhibition zone (clear zone) around the reservoir is measured after incubation. Before incubating, the compounds from the reservoir are commonly allowed to diffuse into the agar medium at a lower temperature for a few hours before inoculation with the test bacteria (Cos et al., 2006). The types of reservoirs used can be filter paper disks placed on top of the agar surface or wells punched into the agar, for example. Advantages of the system include small sample requirements and the ability to test up to six extracts per plate against one microorganism (Hadacek and Greger, 2000). However, a major disadvantage is that this method is not suitable for testing nonpolar samples or samples that are unable to diffuse readily through the agar matrix (Cos et al., 2006).

With dilution methods, the test sample is mixed with a medium (liquid broth or solid agar) inoculated with the test microorganism. Growth of the microorganism after incubation can then be monitored in various ways. In agar dilution methods, the minimum inhibitory concentration (MIC) is the lowest concentration of test compound able to inhibit visible microbial growth. In broth dilution methods, turbidity (measured visually or spectrophotometrically) and redox indicators (commonly a tetrazolium salt, e.g., *p*-iodonitrotetrazolium violet [INT], 3-4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide [MTT], or resazurin) are usually used to detect microbial growth. The presence of "cidal" or "static" effects of a certain concentration of compound or extract can be determined using broth dilution methods (Cos et al., 2006). Minimal bactericidal or fungicidal concentrations (MBC or MFC, respectively) can be detected by plating out samples at inhibitory concentrations onto agar and assessing growth (static) or no growth (cidal) after incubation. Dilution methods are useful in testing both polar and nonpolar extracts or compounds. The microdilution assay, using various growth indicators, including tetrazolium salts, has been successfully used with fast-growing species of mycobacteria, including *Mycobacterium smegmatis*, *M. aurum,* and *M. fortuitum*, and with slow-growing species such as *M. bovis* and *M. avium* (Chung et al., 1995; Franzblau et al., 1998; McGaw et al., 2008).

For quantifying antibacterial activity, Eloff (2000) proposed that the quantity of material extracted from 1 g of dried plant material be divided by the MIC value to give the total activity of the plant. This measure, in milliliters per gram, indicates the largest volume to which 1 g of the extract (containing active compounds) can be diluted and still inhibit growth of the bacterial (or fungal) species under investigation and thus the potency of the extract.

Bioautography is a valuable technique that localizes antibacterial or antifungal activity on a thin-layer chromatographic (TLC) plate. Components of an extract are

developed on a TLC plate using an appropriate mobile phase (i.e., one that separates the compounds adequately but is also relatively volatile so it evaporates rapidly from the plate). A balance needs to be struck between allowing sufficient time prior to bioautography to pass for the eluting solvent to evaporate completely from the TLC plate, but not too much time for the exposed compounds separated on the TLC plate to decompose as a result of exposure to light and oxygen. In agar overlay bioautography (Hamburger and Cordell, 1987; Rahalison et al., 1991), agar medium mixed with bacterial or fungal culture before it solidifies is poured onto the TLC plate and incubated. In a popular method that avoids the difficulties associated with compounds not being able to diffuse into the agar medium from the TLC plate, a suspension of bacteria or fungi in liquid medium is sprayed onto the developed TLC plate. This is termed direct bioautography (Begue and Kline, 1972). After the plate is sprayed with a suspension of a tetrazolium salt such as INT the presence of clear zones of inhibition are visualized against a purple background to indicate microbial growth. Bioautography facilitates bioassay-guided fractionation for the isolation of antibacterial or antifungal compounds, but its use is restricted to those microorganisms that are able to grow rapidly on a TLC plate with the limited amount of nutrients available for growth in the medium that adheres to the surface of the TLC plate. In this regard, using the technique for filamentous fungi is inappropriate.

Selection of test bacterial species to use in a screening procedure is dependent on the purpose of the study. For routine antibacterial screening, the National Committee for Clinical Laboratory Standards (NCCLS, 1990) (Villanova, Pennsylvania, USA) recommended the Gram-positive *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 29213) and the Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). For antifungal screening projects, representatives from the yeasts (e.g., *Candida albicans*), dermatophytic fungi (e.g., *Trichophyton mentagrophytes* and *Epidermophyton floccosum*), and opportunistic filamentous fungi (*Aspergillus niger* and *Fusarium solani*) could be included (Cos et al., 2006). The NCCLS (1990) recommended an inoculum of approximately 10^5 cfu/mL for bacteria, while for yeasts and fungi an inoculum of between 10^3 to 10^4 colony-forming units (CFU)/mL is sufficient for dilution methods (Hadacek and Greger, 2000). An inoculum size that is too low may give false positive results, while a too large inoculum could increase false negatives (Cos et al., 2006).

It has been estimated that about 75% of rural livestock owners in the Eastern Cape province of South Africa use plant-based treatments to treat their livestock (Masika and Afolayan, 2002). When screened against a panel of 10 bacteria and 5 fungi, extracts of *Combretum caffrum*, *Salix capensis,* and *Schotia latifolia* showed good activity against all the Gram-positive bacteria and some antifungal activity (Masika and Afolayan, 2002). Most of the extracts were not active against the Gram-negative bacterial species; interestingly, some water extracts actually promoted fungal growth (Masika and Afolayan, 2002). This may have been due to nutritive sugars, which partition into the aqueous fraction. The organisms used in this study were selected from those generally associated with infections or disease in humans and animals. Different concentrations of each plant extract were mixed with liquid agar at approximately 60°C before being poured into Petri dishes. Solvent was allowed to evaporate overnight from the plates, and bacteria or fungi were inoculated

onto the plates and inhibition of growth observed. It was concluded that the inhibition of growth of Gram-positive bacteria, the Gram-negative *Enterobacter cloacae,* and several fungal species by water extracts of the plants indicated possible broad-spectrum antimicrobial effects of the plants, validating to a degree the traditional use of these plants (Masika and Afolayan, 2002).

Ethnoveterinary plants used to treat infectious diseases in cattle were screened in a broth microdilution assay for antibacterial activity (McGaw, Van der Merwe, and Eloff, 2007) against the organisms recommended for antibacterial testing by the NCCLS (1990). Hexane, methanol, and water extracts were found to be most active against the Gram-positive *E. faecalis* and *S. aureus.* Gram-positive species are known to be more susceptible to antimicrobials than are Gram-negative bacteria owing to differences in the bacterial cell wall composition (Vlietinck et al., 1995). It was reported by McGaw, Van der Merwe, and Eloff (2007) that a third of plant extracts tested had MIC values less than 1 mg/mL, and it was largely methanol extracts that displayed activity.

Ziziphus mucronata (Rhamnaceae) demonstrated excellent antibacterial activity in the preliminary assay (McGaw, Van der Merwe, and Eloff, 2007), and the antibacterial compounds 2,3-dihydroxyl-up-20-en-28-oic acid and zizyberanalic acid were subsequently isolated from the leaves (Moloto, 2004). The first compound was very active against *Staphylococcus aureus*, supporting claims of the efficacy of leaf pastes of *Z. mucronata* for the treatment of bacterial infections in animals and humans.

Bizimenyera et al. (2005) identified substantial antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa* in *Peltophorum africanum* (Fabaceae), also using the broth microdilution method. The root and bark extracts are used by farmers to treat stomach ailments such as diarrhea and dysentery in cattle (Bizimenyera et al., 2005), and the antibacterial activity discovered in extracts of the plant may warrant its use against bacterial infections.

Rhizomes and roots of the popular ethnoveterinary plant *Gunnera perpensa* (Gunneraceae) are used to treat endometritis and retained placenta in cattle and women (Hutchings et al., 1996; Van Wyk, Van Oudtshoorn, and Gericke, 1997), and the possibility that antibacterial effects could be responsible for its activity has been investigated (McGaw et al., 2005; Drewes et al., 2005). *Gunnera perpensa* rhizome extracts showed only slight activity against several Gram-negative and Gram-positive bacterial species (McGaw, Jäger, and van Staden, 2000; McGaw et al., 2005), indicating that antibacterial efficacy probably played a mere supporting role to the known uterotonic activity (Kaido et al., 1997) in the reputed medicinal value of the rhizome. Drewes et al. (2005) isolated 1,4-benzoquinones from the stem and leaves of *G. perpensa*. One of the benzoquinones had significant antimicrobial activity, with MIC = 9.8 µg/mL against *Staphylococcus epidermidis* (Drewes et al., 2005).

Noteworthy antifungal activity in several *Terminalia* species (Combretaceae) was reported by Masoko, Picard, and Eloff (2005) against various morphological forms of fungi, including yeasts (*Candida albicans* and *Cryptococcus neoformans*), molds (*Aspergillus fumigatus*), and thermally dimorphic fungi (*Sporothrix schenckii*). These fungal species were carefully selected to represent a spectrum of clinical isolates of the most common and important disease-causing fungi in animals. From extracts of *Terminalia* leaves prepared using several organic solvents, the acetone extracts were most active.

1.2.4 Antiviral

In vitro antiviral assay methods are often based on the abilities of viruses to replicate in cell cultures. Certain viruses cause cytopathic effects (CPEs) or form plaques in lawns of cells, facilitating detection of antiviral effects of a substance. Inhibition of viral replication can also be discovered by monitoring the presence of viral products, such as viral RNA, DNA, or polypeptides. Virucidal substances inactivate the ability of a virus to be infective extracellularly and find application as broad-spectrum biocides. Antiviral agents are more interesting as candidates for clinical use because they may interfere with some aspect of viral biosynthesis (Cos et al., 2006). Vlietinck and Vanden Berghe (1991) supplied a useful outline of cell-based assays that can be used for antiviral or virucidal evaluation of pure compounds or plant extracts.

Toxicity to the host cell system must be assessed as part of the antiviral investigation. The Selectivity Index (SI) is the ratio of the maximum drug concentration causing 50% (or 90%) growth inhibition of normal cells (CC_{50} or CC_{90}, respectively) and the minimum drug concentration at which 50% (or 90%) of the virus is inhibited (IC_{50} or IC_{90}, respectively). It is essential to gain an indication of cytotoxicity of the test substance as, without this, results do not distinguish between antiviral effect and effect against the host cell system.

The choice of viruses to use in a screening panel should include representatives of DNA viruses and RNA viruses and could include criteria such as their ability to replicate in the same cell culture. In the Phytomedicine Program at the University of Pretoria, we have begun investigating antiviral activity of ethnoveterinary plants against feline herpesvirus type 1 (FHV-1) as an enveloped virus relatively sensitive to environmental influences. Plants with good activity in this preliminary screen are then assayed for activity against more resistant viruses, such as the lumpy skin disease virus.

No reports could be found of EVM plants being tested for antiviral activity, although many publications reported on efficacy of ethnobotanically chosen plants against a number of different viruses (e.g., Kudi and Myint, 1999; Asres et al., 2001; Lamien et al., 2005). To screen for antiviral activity, variations on virucidal assays are available in the ethnopharmacological literature, and these mainly focus on inhibition of viral CPE or plaque inhibition. In the virucidal assays, monolayers of the appropriate host cell type are cultured in 96-well microtiter plates. In the cytotoxicity aspect of the assay, serial dilutions of plant extract are exposed to the cells and incubated for a defined period at 37°C in a 5% CO_2 incubator. Following this, the cells are examined using an inverted microscope for signs of damage. Alternatively, a tetrazolium salt or other color indicator of cell viability may be used to detect cytotoxicity compared to untreated cells. In the antiviral test, serial plant extract dilutions are prepared as for the cytotoxicity assay, but virus is added to the cells. In their study, Kudi and Myint (1999) applied tissue culture medium infective dose ($TCID_{50}$) of 10^5 viral particles (100 µL) to each well. The cultures were incubated for an hour to allow adsorption of viral particles, after which 100 µL per well of plant extract dilutions were added to the wells. The plates were incubated for a certain period to allow development of CPEs, if any, and results compared to the controls

consisting of only cells and cells with virus only. A range of different viruses was used in this method.

In an example of a plaque inhibition assay, Zhang et al. (2007) infected monolayers of host (Vero) cells grown in six-well culture plates with 100–200 plaque-forming units (PFU) of herpes simplex virus (HSV). After incubating the plates for 1 h to allow adsorption of the virus, the inoculum was aspirated from the cells, and the cultures were overlaid with 0.8% methylcellulose in culture medium containing dilutions of the test plant extract. After 3 days of incubation at 37°C, the plates were fixed with formalin, stained with crystal violet, air dried, and the number of plaques counted. Control plates consisted of those without plant extract, and the percentage of plaque formation inhibition was calculated as follows:

$$[(\text{Mean number of plaques in control} - \text{Mean number of plaques in test})/(\text{Mean number of plaques in control})] \times 100$$

1.2.5 Antiprotozoal and Antirickettsial

Babesiosis is a protozoan, tick-borne disease affecting many vertebrate hosts. The rhizome extract, prepared using acetone, of the popular ethnoveterinary medicinal plant *Elephantorrhiza elephantina* was shown to have *in vitro* antibabesial activity (Naidoo et al., 2005). In this test system, *Babesia caballi* cultures (isolated from a horse) were incubated in 24-well culture plates with plant extracts at varying concentrations, together with uninfected blood. Parasite growth inhibition was monitored initially by a change in the color of the culture medium, where inhibited cultures remained bright red while unaffected protozoal cultures turned a dark coffee color. Culture smears were then evaluated using light microscopy to determine the percentage of infected cells. The registered antibabesial drugs diminazene aceturate (Berenil) and imidocarb diproprionate (Forray-65) were included as positive controls. Acetone extracts of *Urginea sanguinea*, *Rhoicissus tridentata,* and *Aloe marlothii* were not active in this assay (Naidoo et al., 2005).

Another important protozoal disease occurring in domestic livestock and chickens is coccidiosis, which results from infection with *Eimeria* or *Isospora* species. Coccidiosis causes losses worth millions of U.S. dollars annually in the poultry industry, resulting from animal mortality or poor productivity as well as costs of treatment (Williams, 1999). The use of plants to combat coccidiosis is an emerging field of investigation as these remedies may function by mechanisms different from those of conventional therapeutic agents. In one such study, four plant extracts with reported antioxidant activity were screened for their anticoccidial activity against an artificially induced mixed *Eimeria* infection in poultry (Naidoo et al., 2008). Orally administered *Combretum woodii* was toxic to the birds at a concentration of 160 mg/kg, while *Tulbaghia violacea* (35 mg/kg), *Vitis vinifera* (75 mg/kg), and *Artemisia afra* (150 mg/kg) produced feed conversion ratios similar to toltrazuril, the positive control, and higher than the untreated

control. *Tulbaghia violacea* significantly decreased the oocyst production in the birds, and it was concluded that antioxidant-rich plant extracts have potential benefits in treating and possibly preventing coccidial infections (Naidoo et al., 2008). The results for extracts of *T. violacea* in particular provide momentum for more detailed investigation of the plant as a potential therapeutic or prophylactic anticoccidial agent.

The antirickettsial activity of *Elephantorrhiza elephantina* and *Aloe marlothii* was evaluated using an *in vitro Ehrlichia ruminantium* culture system (Naidoo, Chikoto, et al., 2006). Acetone extracts of the leaves were incubated with *E. ruminantium* cultures, and their activity was compared to that of oxytetracycline and untreated controls. *Elephantorrhiza elephantina* and *A. marlothii* demonstrated EC_{50} values of 111.4 and 64.5 µg/mL and EC_{90} values of 228.9 and 129.9 µg/mL, respectively, indicating good anti-ehrlichial activity. The EC_{50} and EC_{90} values for oxytetracycline were 0.29 and 0.08 µg/mL, respectively. Naidoo, Chikoto, et al. (2006) surmised that the plant extracts produced their inhibitory activity by a similar mechanism, unrelated to that of the tetracyclines.

1.2.6 Anthelmintic

Helminth parasites of livestock are common in rural areas, and anthelmintic remedies form a major component of EVM, as is the case with human traditional medicine. Laboratory research on anthelmintic activity of plant extracts is constrained by the expense, ethical issues and time associated with performing *in vivo* trials, and the difficulties experienced in maintaining parasitic nematodes in culture systems *in vitro*. A free-living nematode, *Caenorhabditis elegans*, has been used as a model organism in broad screening studies as it is easier and cheaper than using parasitic nematodes (Simpkin and Coles, 1981; Rasoanaivo and Ratsimamanga-Urverg, 1993). Notwithstanding the limitations encountered in extrapolating activity against a free-living nematode to activity against a parasitic species (Geary and Thompson, 2001), most commercially available broad-spectrum anthelmintics demonstrate activity against *C. elegans* (Simpkin and Coles, 1981).

In vitro screening investigations have revealed that many plant extracts show activity against the free-living *C. elegans* nematodes (McGaw, Jäger AK, and van Staden, 2000; McGaw and Eloff, 2005; McGaw, Van der Merwe, and Eloff, 2007). A rapid inhibition assay is easy and simple to perform and entails incubating varying concentrations of plant extracts with nematodes for a defined period of 2 h and scoring the percentage of paralyzed or dead nematodes in comparison to the untreated control (Rasoanaivo and Ratsimamanga-Urverg, 1993). Using this assay, several plant species belonging to the family Combretaceae exhibited interesting anthelmintic activity against *C. elegans* (McGaw et al., 2001). These studies may constitute a first step in validating the use of these plants in treating worm infestations in animals and in humans. In a more complicated screening system that evaluates the ability of the nematodes to grow and reproduce, plant extracts are incubated with nematodes in appropriate culture medium with bacterial and fungal growth inhibitors in 24-well assay plates for 7 days, after which the percentage of surviving nematodes is

compared to that of the control wells (Simpkin and Coles, 1981; McGaw, Jäger AK, and van Staden, 2000).

Plant extracts have also been tested using *in vitro* assays with parasitic nematode eggs and larvae. Egg hatch and larval development inhibition against the two most important livestock nematode parasites *Haemonchus contortus* and *Trichostronglyus colubriformis* by various plant extracts have been reported. In these assays, the nematodes are maintained in monospecifically infected lambs, and eggs are collected from the feces. Test substances incubated with the freshly collected eggs may inhibit hatching in the aptly termed egg hatch assay (Coles et al., 1992). The larval development assay (Coles et al., 1988) detects the ability of plant extracts, or other compounds, to retard development of the eggs into infective larvae. The combination of the two assays can provide a practical indication of anthelmintic activity of plant extracts or pure compounds isolated from the extracts.

Peltophorum africanum is a popular plant for use in treating helminthosis, and the acetone extracts of the leaf, bark, and root have been screened for activity against *H. contortus* and *T. colubriformis* in the egg hatch and larval development assays (Bizimenyera, Githiori, Eloff, et al., 2006; Bizimenyera, Swan, et al., 2006). The extracts all showed activity in the assays at a concentration of 0.2 mg/mL, providing some support for the use of this plant in traditional medicine. Further confirmation of nontoxicity and efficacy is required, particularly *in vivo.* Various animal models have been used to detect anthelmintic effects of plant extracts (Kahiya, Mukaratirwa, and Thamsborg, 2003; Iqbal et al., 2006; Jabbar et al., 2007).

1.2.7 Antitick

Tick-borne diseases are a major source of concern for livestock farmers. Research has been undertaken on the repellent and toxic effects of plant extracts against ticks, with promising results thus far. Nchu (2004) analyzed the repellent effects of extracts of *Allium* species, as well as the direct toxicity, against adults of *Hyalomma marginatum rufipes.* Acetone extracts of *A. porrum* revealed a high repellency index (65–79.48%), and the dichloromethane extract of *A. sativum* was toxic to 100% of ticks within an hour of exposure. *Lippia javanica* and *Tagetes minuta* essential oils had a concentration-dependent effect on the ticks (Nchu, 2004), and *T. minuta* delayed molting to adult stage of 60% of engorged nymphs of *H. m. rufipes* in a growth inhibition bioassay. Thembo (2006) showed that *Senna italica* ssp. *arachoides* ethyl acetate extracts had a concentration-dependent acaricidal effect on *H. m. rufipes.* When *S. italica* ssp. a*rachoides* aqueous extracts were fed to guinea pigs and rabbits, the feeding performance of adult *H. m. rufipes* ticks appeared to be impaired (Thembo, 2006).

Plants are used in many African countries as antitick agents on livestock, and 28 of these plants from Ethiopia showed promising repellency activities against adult *Rhipicephalus pulchellus* ticks, with *Calpurnia aurea* displaying the highest toxicity toward the ticks (Zorloni, 2007). Some plants used in South Africa as traditional arthropocides were screened for antitick effects, with *Eucalyptus globoidea* and *Lavendula angustifolia* emerging as effective tick repellents (Mkolo, 2008).

1.2.8 Antioxidant

The current literature reveals a proliferation of recent articles describing antioxidant activity of plant extracts. It should be kept in mind from the outset that most flowering plants contain some antioxidants, and the activity of a plant against a certain disease generally cannot be explained exclusively in terms of its antioxidant activity (Houghton et al., 2007). Oxidative damage caused by free radicals, or reactive oxygen species, has been implicated in contributing to the progression of a number of diseases, such as cardiovascular disease, many cancers, and diabetes. Various *in vitro* screening systems for antioxidant activity have been described, including those based on chemical reactions, for example, the diphenyl–picrylhydrazyl (DPPH) free-radical scavenging test (Mensah et al., 2004), those involving biological models such as liposomes to mimic cell wall lipids (Dickson et al., 2006), and those involving cells challenged with prooxidants (Mensah et al., 2001).

The TEAC (trolox equivalent antioxidant capacity) assay described by Re et al. (1999) has a major advantage in that it is applicable to both aqueous and lipophilic systems. It is a decolorization assay that measures antioxidant activity in comparison to trolox, a water-soluble vitamin E analogue. This assay begins with generation of the radical monocation 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), or $ABTS^{+}$, a blue-green compound produced by reacting ABTS with potassium sulfate. When the free radical is incubated with antioxidants, these compounds reduce the radical to colorless ABTS, and this reaction depends on the concentration of the antioxidant and the time during which the reaction is allowed to occur. The level of decolorization as percentage inhibition of the free radical is calculated relative to the reactivity of trolox under identical conditions (Re et al., 1999).

The widely used ethnoveterinary plant *Peltophorum africanum* is used to promote well-being and resistance to diseases in cattle, in addition to the previously mentioned use in treating stomach upsets (Bizimenyera et al., 2005). On screening the plant for antioxidant activity using the TEAC assay, it was found that root extracts possessed good antioxidant activity, particularly the ethanol extract of the root. The level of polyphenols in the roots was also high, probably contributing in large part to the overall antioxidant activity (Bizimenyera et al., 2005). In the study of Naidoo et al. (2008), plant extracts with high antioxidant activity were shown to be effective in treating coccidiosis infections in chickens.

After Naidoo et al. (2005) reported that extracts of *Rhoicissus tridentata,* a plant used for the treatment of babesiosis in cattle, were not effective in an *in vitro* antibabesial assay, it was proposed that the reputed efficacy of this plant may result from a reduction in antioxidant cellular injury (Naidoo, Zweygarth, and Swan, 2006). Antioxidant evaluation of acetone extracts of different plant parts revealed good activity in the DPPH assay and in the TEAC assay (value of 2.5). The activity was held to owe in part to the presence of catechin, epicatechin, gallic acid, and epigallocatechin-gallate in the tuber acetone extracts as demonstrated by high-performance liquid chromatographic (HPLC) analysis (Naidoo, Zweygarth, and Swan, 2006). It was concluded that *R. tridentata* might be effective in animals infected with babesiosis by limiting the degree of oxidative cellular injury (Naidoo, Zweygarth, and Swan, 2006).

1.2.9 Anti-inflammatory and Wound Healing

Many disease conditions are associated with excessive inflammation (e.g., arthritis, eczema, and asthma), so assays detecting inhibition of one of the many biochemical processes related to inflammation are commonly used as *in vitro* screens for anti-inflammatory activity (Houghton et al., 2007). These include tests for cyclooxygenase inhibition, lipoxygenase inhibition, inhibition of eicosanoid synthesis, or nuclear factor kappa B (NFκB) production.

Traditional EVM makes use of many preparations to treat wounds in livestock animals as such wounds may lead to more serious problems. Wound healing is a complex combination of processes, including inflammation, cell proliferation, collagen formation, and contraction of the collagen lattice (Houghton et al., 2007). Healing of the wound may be complicated by the presence of microbial infection and destruction of cells and tissues by reactive oxygen species. If a livestock owner uses a particular plant medicine to treat wounds, this preparation may affect one or more of the processes described. Therefore, a battery of *in vitro* tests should be conducted to verify the wound-healing activity of the extract, such as stimulation of fibroblast proliferation, antibacterial activity, and free-radical scavenging effects. Anti-inflammatory activity may provide short-term relief. The development of tests for wound healing, from *in vivo* tests to cell-based systems and chemical reactions and further to investigations into effects on secondary messengers and protein expression, has been described by Houghton et al. (2005).

In an efficacy study of South African plants used for wound healing and against retained placenta, Luseba et al. (2007) discovered that several dichloromethane extracts displayed antibacterial and anti-inflammatory activity. Extracts of *Cissus quadrangularis* stem and *Jatropha zeyheri* root showed selective inhibition against cyclooxygenase-2 in the anti-inflammatory experiments. Even though water is traditionally the most commonly used solvent to prepare medicinal extracts, the activity of organic extracts need not be disregarded. According to Luseba et al. (2007), in the treatment of livestock wounds the whole plant material is often locally applied, and in the case of complaints such as retained placenta, for which the treatment mixtures are given orally (and are unlikely to be filtered), active ingredients may be released.

EVM remedies may in some cases be applied topically to wounds or skin infections, and rodent models to evaluate *in vivo* effects of plant extracts and compounds isolated from them with promising *in vitro* activity have been reported (Kruger, 2004; Masoko, 2006). In these methods, a number of small topical wounds are created on the back of a shaved rat, and if investigating antibacterial or antifungal activity as well as wound-healing effects, a bacterial or fungal culture may be used to infect some of the lesions. Following this, preparations of extract or compound in aqueous cream are applied to the wound, and various parameters such as erythema, exudate, and wound diameter are monitored throughout the duration of the study. Signs of toxicity are noted, and gross pathology is performed on necropsy. Owing to the fact that approximately six wounds can be made on each rat, the rats serve as their own controls to reduce the number of rats used in the study. One wound acts as an untreated control, another is treated with a standard antibiotic, and the remaining wounds are treated with test preparations. To allow this reduction in the number of

animals used, the assumption is made that systemic effects of the topically applied medication are nonexistent.

1.3 TOXICITY STUDIES

Toxicity investigations on EVM plant extracts are necessary, both to evaluate the potential toxic effects toward the animal being treated and to exclude false-positive activity results in antimicrobial assays arising from nonspecific toxic properties. In southern Africa, there is a rich floral diversity, and approximately 600 toxic species are known to occur in this region (Kellerman, Coetzer, and Naudé, 1992). Van der Merwe, Swan, and Botha (2001) commented that side effects and toxicity associated with the medicinal use of plants in EVM were rarely reported, although several plants used have potentially dangerous toxic effects (e.g., *Boophane disticha, Ricinus communis,* and *Solanum* species). The lack of toxic reports was ascribed to the relatively small quantities used in traditional medicines (van der Merwe, Swan, and Botha, 2001). Toxicity is affected in ruminants by the degradation or binding of toxins by the ruminal microflora or the digestive tract, as harmless precursors can be converted to toxic substances or less toxic substances can be changed to more toxic ones (Naudé, Coetzer, and Kellerman, 1992). It is therefore important to evaluate potential toxic effects of EVM remedies, particularly those preferred for oral dosing. A screening regimen that includes assays to test for genotoxicity, cytotoxicity, and *in vivo* toxicity (acute and chronic) is advised. This section is only touched on as other chapters in this volume deal with this aspect in more detail.

Several approaches have been followed to assess toxicity of natural remedies, including testing for genotoxic effects using *in vitro* bacterial and mammalian cell assays such as the Ames test, micronucleus test, and comet assay (Fennell et al., 2004). Luseba et al. (2007) tested dichloromethane and 90% methanol extracts of 12 South African plants used to treat retained placenta and wounds in livestock for mutagenicity and found that none of the extracts was mutagenic in the Ames test using *Salmonella typhimurium* strain TA98 without metabolic activation.

A quick and easy way to gain a preliminary indication of cytotoxicity is to submit extracts to the brine shrimp assay. This assay has been used to detect *in vitro* cytotoxic or pharmacological effects (Solís et al., 1993) as activity in this assay has been correlated with cytotoxicity in a number of cell lines, including 9KB, P388, L5178Y, and L1210 (Meyer et al., 1982; McLaughlin, 1991; De Rosa, De Giulio, and Iodice, 1994; McLaughlin, Rogers, and Anderson, 1998). The brine shrimp assay involves incubating test substances with freshly hatched brine shrimp larvae and detecting percentage mortality of the larvae. A shortcoming of this technique is that it does not account for metabolic activation of the test extracts or compounds, and it is difficult to extrapolate toxicity against a crustacean to mammalian cytotoxicity even though correlations have been noted with cytotoxicity in some cell lines. As an example, McGaw and Eloff (2005) reported that few extracts of plants known to be toxic to livestock were active in the brine shrimp assay. In a later study of plants used to treat cattle for various ailments (McGaw, Van der Merwe, and Eloff, 2007), the lowest IC_{50} value recorded was 0.6 mg mL^{-1}.

Cytotoxicity assays using cell lines are generally relatively easy to perform, although specialized cell culture facilities are required. A range of cells may be used, including continuous commercially available cell lines as well as primary cells derived from animals. Various indicators of cell viability following incubation with plant extracts may be used, including those to detect mitochondrial activity or cellular integrity.

1.4 CONCLUSION

Research on natural products from plants traditionally used to treat animals as well as humans is a key mechanism for identifying new chemical entities that may have interesting biological activity. Isolation of active compounds is not the only useful pathway in medicinal studies. Optimization of extract preparations by removing bulky inactive constituents while leaving behind compounds that may have a synergistic or additive beneficial effect may also lead to useful medications. This could be of particular value when considering primary health care systems in rural areas as well as assisting rural livestock owners in managing disease in their animals. Standardized and formulated plant extracts may be an initiative for developing countries to follow up in originating successful pharmaceutical industries that can compete with Western pharmaceutical companies for the treatment of various diseases, in both humans and animals (Pieters and Vlietinck, 2005). With the current controversy over antibiotic use as growth promoters in production animals, plants with positive effects on the growth and well-being of animals may provide alternatives to be investigated in this lucrative market.

It should be kept in mind that EVM involves a complex system that merges treatment of diseases with herbal and other remedies with management practices such as disease prevention. To meet the aims of validating the uses of plants in EVM and developing and providing more cost-effective veterinary remedies, other factors such as socioeconomic assessments must take place in conjunction with pharmacological evaluations of efficacy and toxicity. Economic considerations must be taken into account; for example, a particular natural remedy may be less effective than a commercial treatment, but a cost-benefit analysis may reveal that using EVM is economically more beneficial. Management and prevention issues are integral to successful animal health care management, and promotion of the use of pharmacologically proven and nontoxic plant medicines is part of this management system.

In this chapter, the emphasis has of necessity been placed on *in vitro* evaluation of biological activity as a result of the shortage of *in vivo* studies concerning EVM remedies. Ethnopharmacological researchers must assimilate the strengths and weaknesses of *in vitro* tests and take into account pharmacokinetic factors, traditional methods of preparation of medicines, the effect of other added substances or adulterants, and the dose showing activity. The use of accepted laboratory testing equivalents to animal studies is ethically and economically desirable. More than one test system related to the disease under investigation should be employed to evaluate biological activity, appropriate test organisms must be used, and activity should ideally be confirmed by *in vivo* tests if sufficiently justified. Standardization of techniques to

improve interlaboratory comparisons of results with different plants analyzed for the same activity is a priority. These methods for evaluating efficacy need to be specific and rapid and not require a large quantity of material. While activity *in vitro* does not necessarily confirm the efficacy and safety of a plant extract, it may provide preliminary indications of the usefulness and potential toxicity of the plant.

Validation is essential to avoid perpetuation of the preconceived notion that traditional medicine is primitive and inherently inferior. Strides have been taken in evaluating the usefulness of human ethnomedicine, and similar action needs to be taken to objectively evaluate EVM to avoid negative stereotypes that obscure the potential benefits to be obtained from traditional ethnoveterinary practices as well as plant-based natural remedies. Investigating the biological activity of ethnoveterinary plants may provide valuable leads for further targeted research that could generate marketable products, whether potentized extracts, isolated compounds, or modified compounds of natural origin. Overall, ethnoveterinary knowledge is an important resource that stands to benefit not only those pastoralists currently making use of traditional remedies to treat their animals, but also researchers seeking innovative and effective treatments for animal and human disease worldwide.

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REFERENCES

Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecouque C, De Clercq E. 2001. Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnobotanically selected Ethiopian medicinal plants. *Phytotherapy Research* 15, 62–69.

Begue WJ, Kline RM. 1972. The use of tetrazolium salts in bioautographic procedures. *Journal of Chromatography* 64, 182–184.

Bizimenyera ES, Githiori JB, Eloff JN, Swan GE. 2006. In vitro activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. *Veterinary Parasitology* 142, 336–343.

Bizimenyera ES, Githiori JB, Swan GE, Eloff JN. 2006. In vitro ovicidal and larvicidal activity of the leaf, bark and root extracts of *Peltophorum africanum* Sond. (Fabaceae) on *Haemonchus contortus*. *Journal of Animal and Veterinary Advances* 5, 608–614.

Bizimenyera ES, Swan GE, Chikoto H, Eloff JN. 2005. Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine. *Journal of the South African Veterinary Association* 76, 54–58.

Borris RP. 1996. Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology* 51, 29–38.

Chung GAC, Aktar Z, Jackson S, Duncan K. 1995. High-throughput screen for detecting antimycobacterial agents. *Antimicrobial Agents and Chemotherapy* 39, 2235–2238.

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ. 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 44, 35–44.

Coles GC, Tritschler JP, Giordano DJ, Laste NJ, Schmidt AL. 1988. Larval development test for detection of anthelmintic resistant nematodes. *Research in Veterinary Science* 45, 50–53.

Cos P, Vlietinck AJ, Vanden Berghe D, Maes L. 2006. Anti-infective potential of natural products: how to develop a stronger in vitro "proof-of-concept." *Journal of Ethnopharmacology* 106, 290–302.

Cowan MM. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12, 564–582.

De Rosa S, De Giulio A, Iodice C. 1994. Biological effects of prenylated hydroquinones: structure–activity relationship studies in antimicrobial, brine shrimp, and fish lethality assays. *Journal of Natural Products* 57, 1711–1716.

Dickson RA, Houghton PJ, Hylands PJ, Gibbons S. 2006. Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamanium* Baill., *Securinega virosa* Roxb. and Wlld. and *Microglossa pyrifolia* Lam. *Phytotherapy Research* 20, 41–45.

Dold AP, Cocks ML. 2001. Traditional veterinary medicine in the Alice district of the Eastern Cape Province, South Africa. *South African Journal of Science* 97, 375–379.

Drewes SE, Khan F, van Vuuren SF, Viljoen AM. 2005. Simple 1,4-benzoquinones with antibacterial activity from stems and leaves of *Gunnera perpensa*. *Phytochemistry* 66, 1812–1816.

Eloff JN. 1998a. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* 64, 711–714.

Eloff JN. 1998b. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60, 1–8.

Eloff JN. 2000. A proposal on expressing the antibacterial activity of plant extracts—a small first step in applying scientific knowledge to rural primary health care in South Africa. *South African Journal of Science* 96, 116–118.

Farnsworth NF. 1984. The role of medicinal plants in drug development. In: Krogsgaard-Larsen P, Brogger Christensen L, Kofold H. (Eds.), *Natural Products and Drug Development*. Munksgaard, Copenhagen, pp. 17–28.

Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM, van Staden J. 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology* 94, 205–217.

Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. 1998. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *Journal of Clinical Microbiology* 36, 362–366.

Geary TG, Thompson DP. 2001. *Caenorhabditis elegans*: how good a model for veterinary parasites? *Veterinary Parasitology* 101, 371–386.

Hadacek F, Greger H. 2000. Testing of antifungal natural products: methodologies, comparability of results and assay choice. *Phytochemical Analysis* 11, 137–147.

Hamburger MO, Cordell GA. 1987. A direct bioautographic TLC assay for compounds possessing antibacterial activity. *Journal of Natural Products* 50, 19–22.

Houghton PJ, Howes M-J, Lee CC, Steventon G. 2007. Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant. *Journal of Ethnopharmacology* 110, 391–400.

Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. 2005. In vitro tests and ethnopharmacological investigations: wound healing as an example. *Journal of Ethnopharmacology* 100, 100–107.

Hutchings A, Scott AH, Lewis G, Cunningham AB. 1996. *Zulu Medicinal Plants: An Inventory*. Pietermaritzburg, South Africa: University of Natal Press.

Iqbal Z, Lateef M, Akhtar MS, Ghayur MN, Gilani AH. 2006. In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *Journal of Ethnopharmacology* 106, 285–287.

Jabbar A, Zaman MA, Iqbal Z, Yaseen M, Shamim A. 2007. Anthelmintic activity of *Chenopodium album* (L.) and *Caesalpinia crista* (L.) against trichostrongylid nematodes of sheep. *Journal of Ethnopharmacology* 114, 86–91.

Kahiya C, Mukaratirwa S, Thamsborg SM. 2003. Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. *Veterinary Parasitology* 115, 265–274.

Kaido TL, Veale DJH, Havlik I, Rama DBK. 1997. Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. *Journal of Ethnopharmacology* 55, 185–191.

Kellerman TS, Coetzer JAW, Naudé TW. 1992. Plant poisonings and mycotoxicoses of livestock in southern Africa. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP (Eds.), *Poisonous Plants: Proceedings of the Third International Symposium*. Iowa State University Press, Ames, IA, pp. 43–54.

Kruger J. 2004. Isolation and characterization of antibacterial compounds from three *Terminalia* species. PhD thesis, University of Pretoria, South Africa.

Kudi AC, Myint SH. 1999. Antiviral activity of some Nigerian medicinal plant extracts. *Journal of Ethnopharmacology* 68, 289–294.

Lamien CE, Meda A, Mans J, Romito M, Nacoulma OG, Viljoen GJ. 2005. Inhibition of fowlpox virus by an aqueous acetone extract from galls of *Guiera senegalensis* J. F. Gmel (Combretaceae). *Journal of Ethnopharmacology* 96, 249–253.

Laupattarakasem P, Houghton PJ, Hoult JRS, Itharat A. 2003. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. *Journal of Ethnopharmacology* 85, 207–215.

Luseba D, Elgorashi EE, Ntloedibe NT, Van Staden J. 2007. Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. *South African Journal of Botany* 73, 378–383.

Luseba D, Van der Merwe D. 2006. Ethnoveterinary medicine practices among Tsonga speaking people of South Africa. *Onderstepoort Journal of Veterinary Research* 73, 115–122.

Masika PJ, Afolayan AJ. 2002. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. *Journal of Ethnopharmacology* 83, 129–134.

Masika PJ, Sonandi A, Van Averbeke W. 1997. Perceived causes, diagnosis and treatment of babesiosis and anaplasmosis in cattle by livestock farmers in communal areas of the central Eastern Cape Province, South Africa. *Journal of the South African Veterinary Association* 68, 40–44.

Masika PJ, Van Averbeke W, Sonandi A. 2000. Use of herbal remedies by small-scale farmers to treat livestock diseases in central Eastern Cape Province, South Africa. *Journal of the South African Veterinary Association* 71, 87–91.

Masoko P. 2006. Characterisation of antifungal compounds isolated from *Terminalia* and *Combretum* species (Combretaceae). PhD thesis, University of Pretoria, South Africa.

Masoko P, Picard J, Eloff JN. 2005. Antifungal activities of six South African *Terminalia* species (Combretaceae). *Journal of Ethnopharmacology* 99, 301–308.

Mathias E. 2004. Ethnoveterinary medicine: harnessing its potential. *Veterinary Bulletin* 74, 27–37.

Mathias E. 2007. Ethnoveterinary medicine in the era of evidence-based medicine: mumbo-jumbo, or a valuable resource? *The Veterinary Journal* 173, 241–242.

McGaw LJ, Eloff JN. 2005. Screening of sixteen poisonous plants for antibacterial, anthelmintic and cytotoxic activity in vitro. *South African Journal of Botany* 71, 302–306.

McGaw LJ, Eloff JN. 2008. Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology* 119, 559–574.

McGaw LJ, Gehring R, Katsoulis L, Eloff JN. 2005. Is the use of *Gunnera perpensa* extracts in endometritis related to antibacterial activity? *Onderstepoort Journal of Veterinary Research* 72, 129–134.

McGaw LJ, Lall N, Hlokwe TM, Michel AL, Meyer JJM, Eloff JN. 2008. Purified compounds and extracts from *Euclea* species with antimycobacterial activity against *Mycobacterium bovis* and fast-growing mycobacteria. *Biological and Pharmaceutical Bulletin.* 31, 1429–1433.

McGaw LJ, Jäger AK, van Staden J. 2000. Antibacterial, anthelmintic and antiamoebic activity in South African medicinal plants. *Journal of Ethnopharmacology* 72, 247–263.

McGaw LJ, Rabe T, Sparg SG, Jäger AK, Eloff JN, van Staden J. 2001. An investigation on the biological activity of C*ombretum* species. *Journal of Ethnopharmacology.* 75, 45–50.

McGaw LJ, Van der Merwe D, Eloff JN. 2007. In vitro anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine. *The Veterinary Journa*l 173, 366–372.

McLaughlin JL. 1991. Crown gall tumours on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening. In: Hostettman K (Ed.), *Methods in Biochemistry, Assays for Bioactivity*, Vol. 6. Academic Press, London, pp. 1–32.

McLaughlin JL, Rogers LL, Anderson JE. 1998. The use of biological assays to evaluate botanicals. *Drug Information Journal* 32, 513–524.

Mensah AY, Houghton PJ, Akyirem GNA, Fleischer TC, Mensah MLK, Sarpong K, Adosraku R. 2004. Evaluation of the antioxidant and free radical scavenging properties of *Secamone afzelii* Rhoem. *Phytotherapy Research* 18, 1031–1032.

Mensah AY, Sampson J, Houghton PJ, Hylands PJ, Westbrook J, Dunn M, Hughes MA, Cherry GW. 2001. Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. *Journal of Ethnopharmacology* 77, 219–226.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Journal of Medicinal Plant Research* 45, 31–34.

Mkolo NM. 2008. Anti-tick properties of some of the traditionally used plant-based products in South Africa. MSc thesis, University of Limpopo, South Africa.

Moloto MP. 2004. Isolation and characterization of antibacterial, anthelmintic, antioxidant and cytotoxic compounds present in *Ziziphus mucronata*. MSc thesis, Medical University of South Africa.

Naidoo V, Zweygarth E, Eloff JN, Swan GE. 2005. Identification of anti-babesial activity for four ethnoveterinary plants in vitro. *Veterinary Parasitology* 130, 9–13.

Naidoo V, Chikoto H, Bekker LC, Eloff JN. 2006. Antioxidant compounds in *Rhoicissus tridentata* extracts may explain their antibabesial activity. *South African Journal of Science* 102, 198–200.

Naidoo V, McGaw LJ, Bisschop SPR, Duncan N, Eloff JN. 2008. The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. *Veterinary Parasitology* 153, 214–219.

Naidoo V, Zweygarth E, Swan GE. 2006. Determination and quantification of the *in vitro* activity of *Aloe marlothii* (A. Berger) subsp. *marlothii* and *Elephantorrhiza elephantina* (Burch.) Skeels acetone extracts against *Ehrlichia ruminantium. Onderstepoort Journal of Veterinary Research* 73, 175–178.

National Committee for Clinical Laboratory Standards (NCCLS). 1990. *Performance Standards for Antimicrobial Disc Susceptibility Tests*, 4th ed. Approved Standard. NCCLS Document M2-A4. Villanova, PA: National Committee for Clinical Laboratory Standards.

Naudé TW, Coetzer JAW, Kellerman TS. 1992. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP (Eds.), *Poisonous Plants: Proceedings of the Third International Symposium*. Iowa State University Press, Ames, IA, pp. 11–20.

Nchu F. 2004. Developing methods for the screening of ethnoveterinary plants for tick control. MSc thesis, Medical University of Southern Africa.

Njoroge GN, Bussmann RW. 2006. Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyus (central Kenya). *Journal of Ethnopharmacology* 108, 332–339.

O'Neill MJ, Lewis JA. 1993. The renaissance of plant research in the pharmaceutical industry. In: Kinghorn, AD, Balandrin MF (Eds.), *Human Medicinal Agents from Plants*. ACS Symposium Series 534. American Chemical Society, Washington, DC, pp. 48–55.

Pieters L, Vlietinck AJ. 2005. Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds? *Journal of Ethnopharmacology* 100, 57–60.

Rahalison L, Hamburger M, Hostettman K, Monod M, Frenk E. 1991. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. *Phytochemical Analysis* 2, 199–203.

Rasoanaivo P, Ratsimamanga-Urverg S. 1993. *Biological Evaluation of Plants with Reference to the Malagasy Flora*. Madagascar: Napreca, pp. 9–43, 72–83.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26, 1231–1237.

Simpkin KG, Coles GC. 1981. The use of *Caenorhabditis elegans* for anthelmintic screening. *Journal of Chemical Technology and Biotechnology* 31, 66–69.

Solís PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. 1993. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica* 59, 250–252.

Thembo MK. 2006. The anti-tick effects of *Senna italica* subsp. a*rachoides* extracts on adults of *Hyalomma marginatum rufipes*. MSc thesis, University of Limpopo, South Africa.

Van der Merwe D, Swan GE, Botha CJ. 2001. Use of ethnoveterinary medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the North West Province of South Africa. *Journal of the South African Veterinary Association* 72, 189–196.

Van Wyk B-E, Van Oudtshoorn B, Gericke N. 1997. *Medicinal Plants of South Africa*. Pretoria, South Africa: Briza.

Vlietinck AJ, Vanden Berghe DA. 1991. Can ethnopharmacology contribute to the development of antiviral drugs? *Journal of Ethnopharmacology* 32, 141–153.

Vlietinck AJ, Van Hoof L, Totté J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwami J. 1995. Screening of a hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of Ethnopharmacology* 46, 31–47.

Williams RB. 1999. A compartmentalized model for the estimation of the cost of coccidiosis to the world's chicken production industry. *International Journal of Parasitology* 29, 1209–1229.

Zhang Y, But PP, Ooi VE, Xu H, Delaney GD, Lee SHS, Lee SF. 2007. Chemical properties, mode of action, and in vivo anti-herpes activities of a lignin-carbohydrate complex from *Prunella vulgaris*. *Antiviral Research* 75, 242–249.

Zorloni A. 2007. Evaluation of plants used for the control of animal ectoparasitoses in southern Ethiopia (Oromiya and Somali regions). MSc thesis, University of Pretoria, South Africa.

2 Logistical and Legal Considerations in Ethnoveterinary Research

Mary Chikombero and Dibungi Luseba

CONTENTS

2.1 INTRODUCTION

Globalization has brought new challenges to the protection of biodiversity. The unprecedented loss of biodiversity in the past few decades due to industrial exploitation and overharvesting has raised global concerns. The manner in which bioprospecting, particularly among indigenous people and the use of traditional knowledge, has been carried out in the past has also been heavily criticized, and such criticisms have centered around allegations of biopiracy, unfair distribution of benefits, illegal appropriation of traditional knowledge, and unethical conduct when it comes to filing and claiming patents on indigenous biological resources.

To tackle these problems, various legal regimes have been instituted at international, regional, and national levels in a bid to promote the sustainable utilization of biodiversity, including medicinal indigenous plants. This chapter discusses plant and information gathering and then outlines the concept of access and benefit sharing (ABS) as one of the approaches adopted at the international level, through the Convention on Biological Diversity (CBD), to protect biodiversity. Some of the

challenges of implementing ABS regulations on the local level are also identified. Intellectual property (IP) rights issues and the World Trade Organization (WTO) agreement are also discussed since the proper implementation of the CBD also has an impact on IP rights. Relevant legislation from South Africa, India, and Costa Rica regulating ABS is given as case studies of national laws that have included the CBD provisions/principles.

2.2 MEDICINAL PLANT GATHERING AND STORAGE

People have used ethnoveterinary medicines (EVMs) for generations, and many reasons have been advanced regarding why the practice continues. Farmers claim that medicinal plants are more efficacious than pharmaceuticals for chronic pathologies. They are reputed to have no side effects, and no withdrawal periods for consumption of meat from treated animals are needed since the plants are thought to be nontoxic. In general, ethnoveterinary products are used after conventional pharmaceutical medicines, for chronic cases, have proved ineffective (Luseba and Van der Merwe, 2006).

Traditional knowledge is passed orally from one generation of knowledge holders to the next both formally and informally. In this form of knowledge transmission, plant names that are different from the common names may be used to protect privileged knowledge. Uninformed persons may not be familiar with the nomenclature that traditional healers and knowledge holders use and will therefore not be able to collect medicinal plants by themselves (Reyneke, 1971). Social conventions also control the collection of indigenous plant material to some extent. For example, the felling of fruit trees such as *Sclerocarya birrea* (Marula tree) is often prohibited; the gathering of medicinal plants may be seasonally restricted; and the vegetation around cemeteries may be protected by various taboos (Cunningham, 1988).

In general, traditional healers are not consulted for animal health care. They may be consulted to find lost animals or for advice and medicines to protect animals against witchcraft and ill winds. Most of the farmers learn about traditional treatments from older family members and other farmers. There is little reference to ancestral guidance as seen in human traditional medicine. Farmers are interested in knowing the medicines used by others, and they are willing to share the information. This is contrary to traditional healers, who tend to keep their knowledge to themselves as it may be their only source of livelihood.

Plant parts that are harvested include roots, tubers, bark, leaves, flowers, fruits, seeds, gums, and nectar (Van Wyk, Van Oudtshoorn, and Gericke, 1997). The pharmaceutical and toxicological effects of different parts of a plant may differ substantially (Iwu, 1993; Van Wyk, Van Oudtshoorn, and Gericke, 1997). Plant parts that tend to be constantly available such as roots and bark are used more often than plant parts such as seed or leaves, which may be seasonal (Iwu, 1993). However, the harvesting of roots frequently destroys plants (Van Wyk, Van Oudtshoorn, and Gericke, 1997). The same applies to overharvesting of bark, while collection of leaves, fruits, seeds, and gum is usually less destructive (Cunningham, 1990).

Medicinal plants are used either fresh or after a period of storage. Plant material is usually stored in a dried form. It may be cut into slices to facilitate drying. Dried material is sometimes powdered before storage. Plant material is stored in bags,

newspaper, glass jars, and cans. Stored plants should be protected from exposure to sun, water, dust, wind, and contact with strangers. Plant material intended for sale in markets is often tied into bundles (Van Wyk, Van Oudtshoorn, and Gericke, 1997). However, ethnoveterinary herbal materials are often used fresh because they are collected when necessary (Luseba and Van der Merwe, 2006).

Rapid rural appraisal (RRA) methods are preferably used when gathering information from communities (Beebe, 1995). It is important to organize meetings with traditional leaders and the state veterinary service officers at the onset to explain the purpose of the research. Oral interviews can thereafter be conducted with farmers at dipping tanks in groups or individually. It is important to interview traditional healers and herbalists since their knowledge is rarely shared with farmers. In most cases, the interviews are conducted through a translator, who may be one of the local animal health officers. A general feedback session should be organized to correct, harmonize, and share the information among farmers. Key areas of investigation and discussions are the farmer's socioeconomic profile, animal husbandry, and local knowledge of animal health care (ethnoetiology, ethnodiagnostics, treatments, and disease control). Detailed information on plants used is recorded, including local names of the remedies, indications, preparations, routes of administration, and dosage if applicable.

Plants should be collected under the guidance of the respondents. Botanical data should be collected using a collection form. Notes are also taken from discussions with respondents. Pictures of the plants are recorded with a digital camera, and precise coordinates of the locations taken with a global positioning system instrument, if possible. Two to three specimens of each plant species are collected, labeled, and pressed for voucher specimen. If a qualified plant taxonomist is not involved in the collection, a specimen needs to be sent to a specialized botanical institution for identification. This is important because a single plant species may have different common or local names, or the same name is given sometimes to plants of different genera or species. Approximately 2 kg fresh plant materials are usually collected; dried under the shade, ground, and stored in darkened glass jars to prevent oxidation; and kept for subsequent laboratory investigations (Luseba and Van der Merwe, 2006). Special attention should be given to succulent plants because they are prone to contamination by fungi. Freeze-drying or drying in autoclave at 40% has been suggested in the case of succulents (e.g., Aloe species).

2.3 DATA ANALYSIS

Data can be analyzed by pairwise and matrix ranking. Statistical measures of central tendency, dispersion, and percentages are computed using appropriate statistical packages, such as the Statistical Package for Social Sciences (SPSS Inc.). Statistical methods can also be used to identify plants used most frequently in veterinary medicine, based on the frequency of association of a particular or perceived medicinal value (botanical consistency) and the frequency of a particular plant species associated with or used to treat a particular disease (Matekaire and Bwakara, 2004). This is referred to as *consistency of veterinary usage*. There is consistency when the same plant genus or family is mentioned at least twice for treating the same illness.

2.4 THE CONVENTION ON BIOLOGICAL DIVERSITY

At the international regulatory level, countries have negotiated the CBD, which was concluded at Rio de Janeiro on June 5, 1992. The CBD is a remarkable framework instrument that lays down broad goals, key objectives, and general principles to be implemented by contracting parties through measures at the national level. The three objectives of the CBD are stated in Article 1 as the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of the benefits arising from the utilization of genetic resources. The CBD has been ratified by 191 countries; notably, the United States has signed but has not ratified the treaty (Cordell, 2000).

The CBD sought to reconcile the diverging interests between states with rich biological diversity (mainly the developing countries of the global south) and states with advanced technology (industrialized nations) by introducing the concept of ABS. Through the principle of common but differentiated responsibilities, contracting parties (signatories to the convention) agreed to come together to conserve biological diversity and to negotiate its access. In the context of the CBD, *access and benefit sharing* is a phrase used to describe access granted by a contracting party of the CBD to its genetic resources to another contracting party to the CBD, with such access on mutually agreed terms (MAT) and subject to fair and equitable sharing of the results and benefits of any research carried out on the resources. The ABS provisions of the CBD have caused controversial debates between developing and developed countries and among advocacy groups, including indigenous and local communities, business, and industry (Siebenhuner and Suplie, 2005). Further, the progress toward achieving the objectives of the CBD has been painfully slow as many countries still have to pass national legislation to give effect to the spirit of the convention.

Regulating ABS issues is rather complex and challenging, and in most cases it is an area that is not easily understandable as it involves a milieu of socioeconomic, legal, scientific, and environmental issues. Three regional groups—the Association of Southeast Asian Nations (ASEAN), the Andean Pact (comprising Bolivia, Colombia, Ecuador, Peru, and Venezuela), and the Organization of African Unity (OAU)—have taken the approach of preparing guidelines for member states to encourage regional consistency in approaching ABS regulation and to help their member states in preparing the complex legislation required (International Development Research Center [IDRC], 2004).

The ASEAN has the ASEAN Framework Agreement on Access to Biological and Genetic Resources (draft text; ASEAN, 2000) while the Andean Pact has Decision 391 on Common Regime on Access to Genetic Resources (Andean Community Commission, 1996). For its part, the OAU (now the African Union) has the African Model Legislation for the Protection of the Rights of Local Communities, Farmers and Breeders and for the Regulation of Access to Biological Resources (OAU, 2000). The regional guidelines set minimum standards for member state domestic laws and have taken different approaches to regulating the ABS area.

In addition to the regional efforts, there is another international initiative that is aimed at assisting with the implementation of the ABS provisions of the CBD. This is the *Bonn Guidelines on Access to Genetic Resources and Fair and Equitable*

Sharing of Benefits Arising Out of Their Utilization adopted in May 2002 (CBD Secretariat, 2000). These are, however, not binding as they are merely guidelines; they are voluntary and flexible and were designed mainly to facilitate the development process of national ABS laws and policies. The guidelines outline the roles and responsibilities of users and providers of genetic resources and encourage stakeholders to use a bilateral approach to facilitate ABS goals (Carrizosa et al., 2004). The key issues that are contained in the Bonn guidelines include advice on:

1. Involvement of relevant stakeholders and capacity building
2. Steps to be taken in the ABS negotiating process
3. Elements of prior informed consent (PIC)
4. Monetary and nonmonetary benefits and incentives
5. National monitoring and reporting and accountability

2.4.1 Access and Benefit Sharing

Historically, genetic resources were regarded as a "common heritage" of humankind that belonged to the public domain and could not be owned by a single group (Soejator et al., 2005). The advances in biotechnological research and the rise of the concomitant IP over discoveries from living material have contributed to a change in the customary treatment of genetic resources as a common good (Van Overwalle, 2005). Article 15(1) of the CBD recognized the sovereign rights of member states over their natural resources and gives authority to determine access to genetic resources to national governments subject to national legislation (CBD, 1992). Member states are therefore supposed to protect their genetic resources and accompanying traditional or indigenous knowledge by enacting appropriate national ABS legislation. Access to genetic resources is, however, subject to PIC, a fair and equitable sharing of benefits arising from commercial or other utilization of said resources. Access and transfer of relevant technology, which is subject to patents and IP rights, should be provided on terms that recognize and offer adequate and effective protection of such rights.

2.4.1.1 The Principles of ABS

The principles of ABS center around negotiations and agreement on PIC, MAT and benefit sharing (BS). The CBD stipulated that the granting of access to genetic resources shall be on MAT and subject to PIC of the country of origin. The CBD also recognized the important role of indigenous and local communities in conserving and sustainably using biological diversity and stated that the benefits arising from their knowledge and innovation should be equitably shared.

The regional and national procedures are naturally different, but they all aim to achieve the same goals of conservation, sustainable use of resources, and fair compensation to access. For example, the African Model Legislation for the Protection of the Rights of Local Communities, Farmers and Breeders and for the Regulation and Access to Biological Resources of the OAU provides that any access to biological resources and knowledge or technologies of local communities in any part of the country shall be subject to an application for the necessary PIC and written